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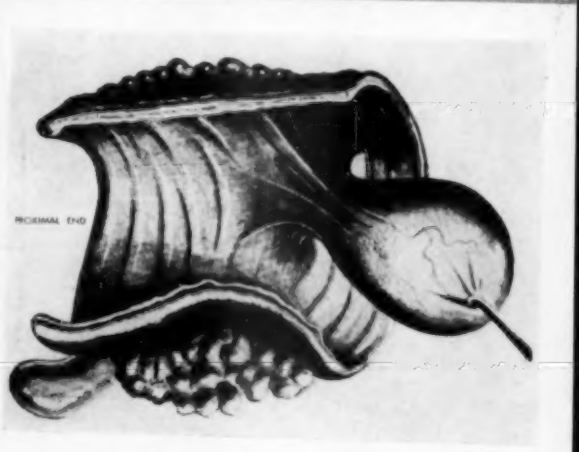

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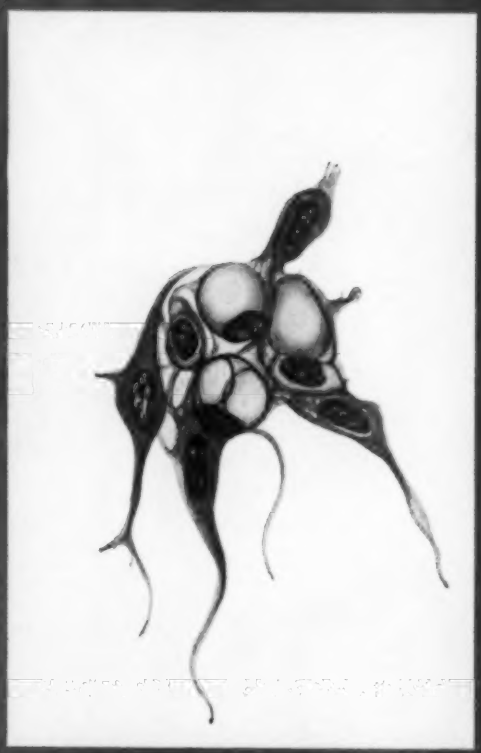

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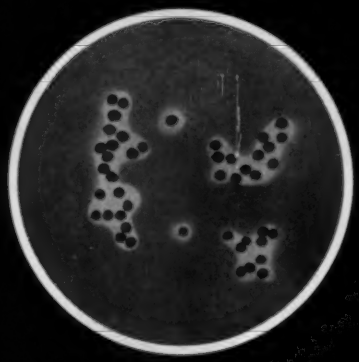

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A Study of the Lymphomas

I. Distribution and Incidence

THEODORE A. THORSON, M.D.
and
DAVID V. BROWN, M.D., Seattle

Over the past quarter-century many significant studies relating to the lymphomas have appeared in the literature.* They have been concerned in detail with problems of morphology, histogenesis, and classification but only to a minor extent with incidence and prognosis. This emphasis on morphological differences has been a necessary consequence of the selective nature of the studies published, most authors having reported on series of collected cases or on cases from their own hospital experience. To answer questions bearing on incidence and prognosis, studies of a somewhat different nature are desirable.

As pointed out by Dunn [8] and as Gilliam [9] has recently reaffirmed, use of the word "incidence" in the recording of disease experience carries with it the implication of risk. The recording of patient-age-distribution data alone, without regard to corresponding data on the population from which the cases have arisen, may give an erroneous impression of the predilection of a given disease for different age groups. As Gilliam [9] stated in his study of the incidence of leukemias and lymphomas,

> To determine the age, sex and race selection (incidence) of these diseases, with full confidence in adequacy of their classification, will require *a cooperative study designed to apply uniform diagnostic techniques to all cases occurring in some definable population such as a large city or a state.*† Data derived from individual hospitals or from the literature summations are generally inadequate for this purpose.

The present study reports on the incidence of the lymphomas, and we feel that it largely fulfils Gilliam's criteria by virtue of the unique nature of the Washington State Tumor Registry ‡ from which all cases were drawn. The study stresses the distinction between age distribution and age incidence. Age distribution shows only the relative frequency with which any given disease is apportioned throughout the various age groups in the general population. Age incidence, on the other hand, relates the number of cases in each age group to total number of people living in that same age group. Hence, incidence serves as a measure of disease selection or "risk" and is expressed as number of cases per unit of population. Although the total number of cases in any one category herein reported is not large, their study in relation to the population background from which they arose may, nonetheless, help clarify some of the incompletely understood problems of incidence of these diseases.

Submitted for publication July 30, 1955.

From the Department of Pathology, University of Washington School of Medicine; Washington State Tumor Registry, and Laboratory Service, Veterans Administration Hospital, Seattle.

* References 1 through 7.

† Italics supplied.

‡ References 10 and 11.

TABLE 1.—*Classification Adopted in the Study*

Diagnostic Category Selected by Authors	"Equivalent" Terms Used by Submitting Pathologists
Follicular lymphoma	Follicular lymphoblastoma Brill-Symmers' disease
Lymphocytic sarcoma	Lymphocytic malignant lymphoma Lymphocytoma Lymphoblastic malignant lymphoma Lymphoblastoma
Reticulum cell sarcoma	Hodgkin's sarcoma Stem cell malignant lymphoma Clasmatocytic malignant lymphoma
Hodgkin's disease	Hodgkin's granuloma (lymphoma) Lymphogranulomatosis

MATERIALS AND METHODS

This report represents an analysis of the morphological features and clinical records of the 334 cases of lymphoma appearing among 19,939 total accessions in the Washington State Tumor Registry. The cases were collected from the entire state of Washington during the five-year period 1947-1952, for which the population of the state is known. It is reliably estimated that 85% of all new cases of neoplastic disease coming to the attention of physicians in the state during this period were submitted to the Registry for review. Cases were actually contributed by 1313 of the some 2100 physicians practicing in the state of Washington at this time.

Each case comprised representative stained slides, a copy of the submitting pathologist's original report, a clinical data sheet, and a final registry diagnosis determined by the board of pathologists constituting the Registry staff. All cases coded as lesions of lymphoid tissues were reviewed by us independently and without knowledge of the previously recorded diagnoses. The cases were then grouped according to the classification shown in Table 1, which in general employs the terminology recommended by the Committee for Clarification of Cells and Diseases of the Blood and Blood-Forming Organs.[12]

Operation of the Registry has included an active follow-up program. Routine inquiries were sent to all contributing physicians at the end of a one-year period and currently are being sent again, five years after the case was originally submitted. In questionable cases results of the follow-up have been utilized, whenever possible, to help establish a definitive diagnosis.

Pertinent population statistics were furnished by the Bureau of Census of the Department of Commerce.[13] The population of the state of Washington for 1950, a median year of the study period, was 2,378,963. This total population was subdivided by the Census Bureau into five-year age groups, making possible the calculation of incidence per 100,000 for each of the lymphomas by decade.

TABLE 2.—*Distribution of Registry Accessions, Showing Relative Frequency of Lymphomas*

Diagnostic Category	No. of Accessions	Per Cent of Accessions
Total accessions	19,939	100.0
Non-neoplastic	1,403	7.0
Neoplastic	18,536	93.0
Benign neoplasms	1,952	10.5
Malignant neoplasms	16,584	89.5
Lymphomas	334	2.0
All other malignant neoplasms	16,250	98.0

TABLE 3.—*Distribution of Lymphomas*

Diagnostic Category	No. of Accessions	Per Cent of Total
Hodgkin's disease	135	40.4
Lymphocytic sarcoma	103	30.8
Reticulum cell sarcoma	65	19.5
Follicular lymphoma	31	9.3
Total	334	100.0

RESULTS

Table 2 shows the distribution of all Registry accessions. Although each case submitted was known or suspected to be a neoplasm on clinical or pathological grounds, it can be seen from Table 2 that 7% of the accessions were eventually regarded as non-neoplastic. Of the 18,536 neoplasms remaining, 10.5% proved to be benign. Finally, as indicated by Table 2, the 334 cases of lymphoma made up 2% of all malignant neoplasms.

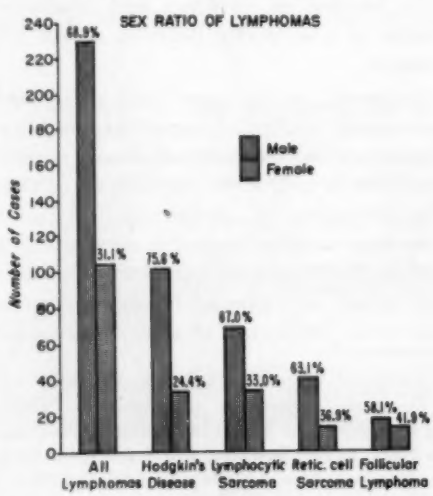

Fig. 1.—Sex ratio of lymphomas.

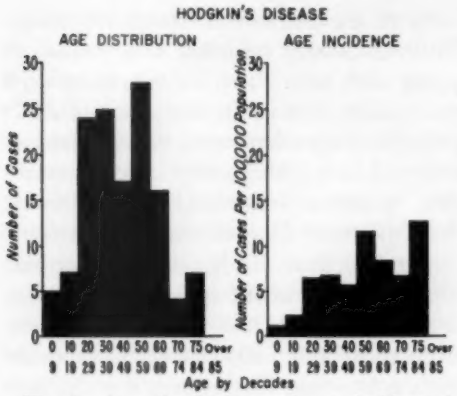

Fig. 2.—Age distribution and age incidence in Hodgkin's disease.

These 334 lymphomas were finally selected only after repeated examination of 813 accessions originally coded as neoplasm involving the lymphoid tissues. The remainder were non-neoplastic or metastatic lesions.

In Table 3 the 334 lymphomas found in the Registry have been grouped by diagnosis. Though correlations with studies by previous authors are difficult because of differences in terminology and classification employed, some comparisons can be made, particularly in the case of Hodgkin's disease and in follicular lymphoma. The percentage (40.4) of Hodgkin's disease in our material is somewhat higher than that reported by Gall and Mallory [3] (31.2), and substantially higher than that found in a comparable study by Hellwig [4] (24.5). The percentage (9.3) recorded by us as follicular lymphoma agrees closely with that (6.8) reported by Gall and Mallory, though it is somewhat smaller than the percentage (16.8) reported by Hellwig.

In Figure 1 the sex ratio for all lymphomas and for each subgroup is shown graphically. The over-all ratio of males to females is 2.2 : 1, which is identical with that reported by Gall and Mallory.[3] However, while the latter investigators found a similar sex ratio (approximately 2 : 1) for Hodgkin's disease, our figures show a 3.1 : 1 preponderance of males. Gall and Mallory indicated a sex distribution ratio of 1 : 1 for follicular lymphoma which approximates the 1.4 : 1 male preponderance which we found. The ratios for reticulum cell sarcoma and for lymphocytic sarcoma in the present study are 1.7 : 1 and 2.0 : 1, respectively, with males predominating in each instance. Here, comparisons with other series are impossible because of the differences in classification mentioned above.

Age-sex distribution data for all cases are summarized in Table 4, where the number of lymphomas in each category have been tabulated by decade. All ages refer to the patient's age at the time a diagnosis of lymphoma was made. It is recognized that the disease may have actually existed for a variable interval before the time of initial diagnosis. The data in Table 4 are shown graphically and in more detail in Figures 2 through 6, where disease distribution is contrasted with disease incidence for all lymphomas and for each individual type.

Figure 2 serves to illustrate the way in which misconceptions may have arisen through failure to differentiate between age distribution and age incidence. Here, in relation to Hodgkin's disease, the graph for age distribution is consistent with the generally accepted idea of peak occurrence in the young adult and middle years with relatively few

TABLE 4.—*Age-Sex Distribution of Lymphomas*

	Decade of Life																					
	0-9		10-19		20-29		30-39		40-49		50-59		60-69		70-74		75-84		Over 85		Total*	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Hodgkin's	4	1	6	1	15	9	18	7	16	1	24	4	10	6	3	1	5	2	0	0	101	32
Lymphocytic sarcoma	3	4	6	0	3	2	6	1	7	5	14	11	13	6	9	1	5	3	0	1	66	34
Reticulum cell sarcoma	1	1	2	0	2	0	3	4	4	2	7	2	14	6	5	7	3	2	0	0	41	24
Follicular lymphoma	0	0	2	0	1	1	3	2	1	1	4	4	5	4	0	1	0	0	0	0	16	13
All lymphomas	8	6	16	1	21	12	30	14	28	9	49	21	42	22	17	10	13	7	0	1	224	103

* Note: Seven cases in the study were deleted from the above Table and from Figures 2 through 6 because their ages were not obtainable.

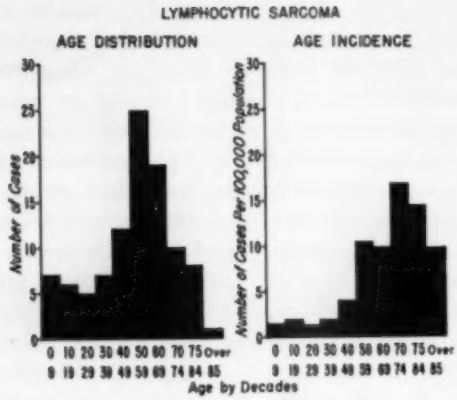

Fig. 3.—Age distribution and age incidence in lymphocytic sarcoma.

cases occurring in the older and younger age groups. This graph of distribution, however, should be contrasted with the graph of incidence, where the number of cases of Hodgkin's disease are shown in relation to the total number of "susceptibles" living in each decade of life. Such a comparison immediately indicates that while Hodgkin's disease does indeed have a relatively high incidence among young adults, the incidence actually appears to increase rather steadily from youth to old age, with the peak being achieved only in the decade 75-84 years. The oldest patient was an 83-year-old man. In our experience the incidence of Hodgkin's disease is low in the first two decades of life, our youngest case occurring in a 6-year-old boy.

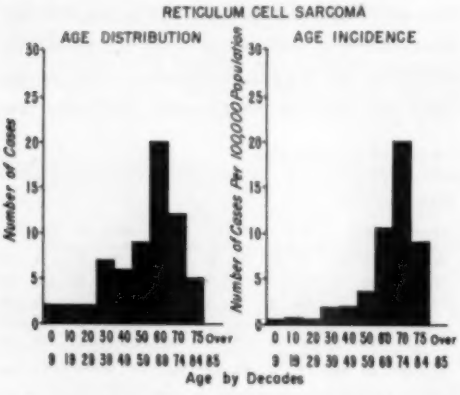

Fig. 4.—Age distribution and age incidence in reticulum cell sarcoma.

As to sex distribution, Hodgkin's disease has been widely regarded as a disease of young adult men. From Table 4, however, it can be seen that in the age group of 20-29 years the preponderance of males to females is only 1.7 : 1, with 9 out of 24 cases occurring in young females. In the following decade, 7 out of 25 cases occurred in females.

Turning next to lymphocytic sarcoma (Figure 3), it would appear from the age distribution graph that this disease is relatively common in early childhood. From the Figure, however, it is evident that the incidence of lymphocytic sarcoma is in reality low in the early decades. A sharp rise is

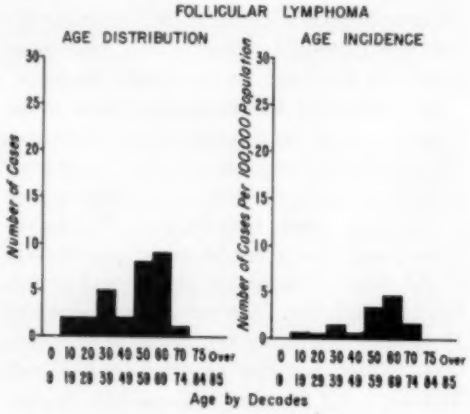

Fig. 5.—Age distribution and age incidence in follicular lymphoma.

seen in the decade of 50-59 years, the disease reaching its peak incidence in the five-year age group of 70-74 years. The only patient in the entire study who reached 85 years of age or over was an 88-year-old woman with lymphocytic sarcoma.

The generally accepted view that reticulum cell sarcoma is seen predominantly in the later decades of life is substantiated by the age distribution graph of Figure 4. The age incidence graph serves as final confirmation, since despite the fact that the largest number of "susceptibles" are in the younger age groups the great majority of cases occurred in the depleted decades of advancing age. As

in the case of lymphocytic sarcoma the peak incidence is seen in the five-year age group of 70-74 years.

The total number of cases of follicular lymphoma in the present series is small. Our experience, however, would appear to correspond to that of Jackson and Parker,[5] Gall and Mallory,[3] and others who found this disease rare in the first decade of life and uncommon in the early decades. The youngest case in the present series was an 11-year-old boy. On the other hand, only one case occurred in a patient over 70 years of age, that in a 72-year-old woman. Here again, as in the case of reticulum cell sarcoma, the incidence graph confirms the generally held view that follicular lymphoma is a disease of late adult life with its greatest incidence in patients aged 50-70 years (Fig. 5).

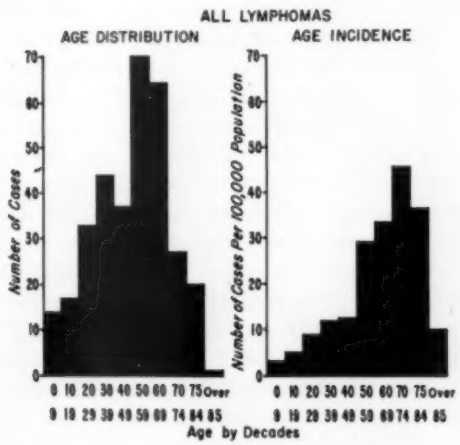

Fig. 6.—Age distribution and age incidence of all lymphomas.

Figure 6 represents a composite distribution-incidence graph of all the lymphomas in the series. Although the greatest number of our cases arose from patients in the decade of 50-59 years, the graph of incidence shows that the risk of incurring some one of the lymphomas becomes progressively greater throughout all of life until a peak is reached in the five-year age group of 70-74 years. This high incidence is essentially sustained throughout the decade of 75-84 years, beyond which little data are available.

SUMMARY AND CONCLUSIONS

A total of 334 lymphomas was found in a study of the 19,939 cases accessioned in the Washington State Tumor Registry during the five-year period 1947-1952. These have been classified according to the recommendations of the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs. The sex distribution ratios for the lymphomas have been determined, and in each instance a male preponderance has been found. This is most apparent in Hodgkin's disease and least evident in follicular lymphoma.

The importance of distinguishing age distribution, often erroneously reported as incidence, from true age incidence which relates disease distribution to population and time is discussed. Our study shows that the lymphomas all have their greatest incidence in the older age groups. Hodgkin's disease varies from the general pattern in that it has a relatively high incidence in the early decades. Here too, however, the incidence becomes progressively greater with advancing age.

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Familial Cardiac Glycogen Storage Disease

Associated Hereditary Maternal Diabetes Mellitus and Obesity

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Von Gierke [1] established the clinicopathologic basis for the hepatic or hepatorenal form of glycogen storage disease in 1929, and about 300 cases of this type have been reported. The cardiac form of glycogen storage disease was described by Bischoff,[2] Putschar,[3] and Pompe [4] in 1932 and 1933, and about 40 examples have been recorded. The various names used in these reports include von Gierke's disease, glycogen disease, glycogen storage disease, glycogenosis, thesaurismosis glycogenica, hepatomegalia glycogenica, hepatonephromegalia glycogenica, and cardiomegalia glycogenica. Most authors have reviewed the preceding literature and described from one to four new cases. Therefore many excellent reviews have appeared.* Di Sant'Agnese, Andersen, Mason, and Bauman [5] analyzed all of the cases reported to 1950 and concluded that in only 34 cases of the hepatic type and in only 12 cases of the cardiac form was there sufficient evidence for a positive diagnosis of glycogen storage disease. They used rigid criteria, including enlargement of involved organs by excessive storage of a glycogenous substance which resisted breakdown. Since 1950 a few more authentic cases of cardiac glycogen storage disease have been reported.

The clinical and pathologic manifestations of glycogen storage disease have varied so greatly that serious doubt has prevailed as to the existence of a single cause or of a single disease entity. Glycogen storage disease has been characterized by an accumulation of a peculiarly stable glycogen in an enlarged liver (von Gierke's disease) or in an enlarged heart, although various other organs also may have been involved. Clinically, the patients with hepatic involvement show a severe hypoglycemia which does not improve with administration of epinephrine. Patients with cardiac involvement show no abnormalities in blood glucose levels, but they usually die of sudden cardiovascular failure within the first 18 months of life.

Two examples of the cardiac form of glycogen storage disease will be presented in this report. In addition to the extreme rarity of this disease, these cases are unique in several respects. First, they occurred in a family with hereditary diabetes mellitus. The hepatic form has been described [10] in connection with hereditary diabetes, but this association has not been recorded hitherto for cardiac glycogen storage disease. It is true that but few thorough studies of families have been presented. Second, when siblings have been affected heretofore, they have been of the same sex. This has led some investigators [16] to postulate a sex linkage. The cases described here were of different sex. Third, because of the great interest in glucagon,† a hyperglycemic glycogenolytic substance supposedly produced by alpha cells ‡ of the islets of Langerhans, detailed study of the pancreas has assumed special importance. Reports of the results of special granule stains of pancreatic islets in cases of glycogen storage disease have not been found in the literature, except for a brief statement about a preponderance of beta cells in hyperplastic islets in a case, reported by Wachstein,[24] in which the special staining apparently was done by Gomori. A detailed analysis of the pancreatic islets is

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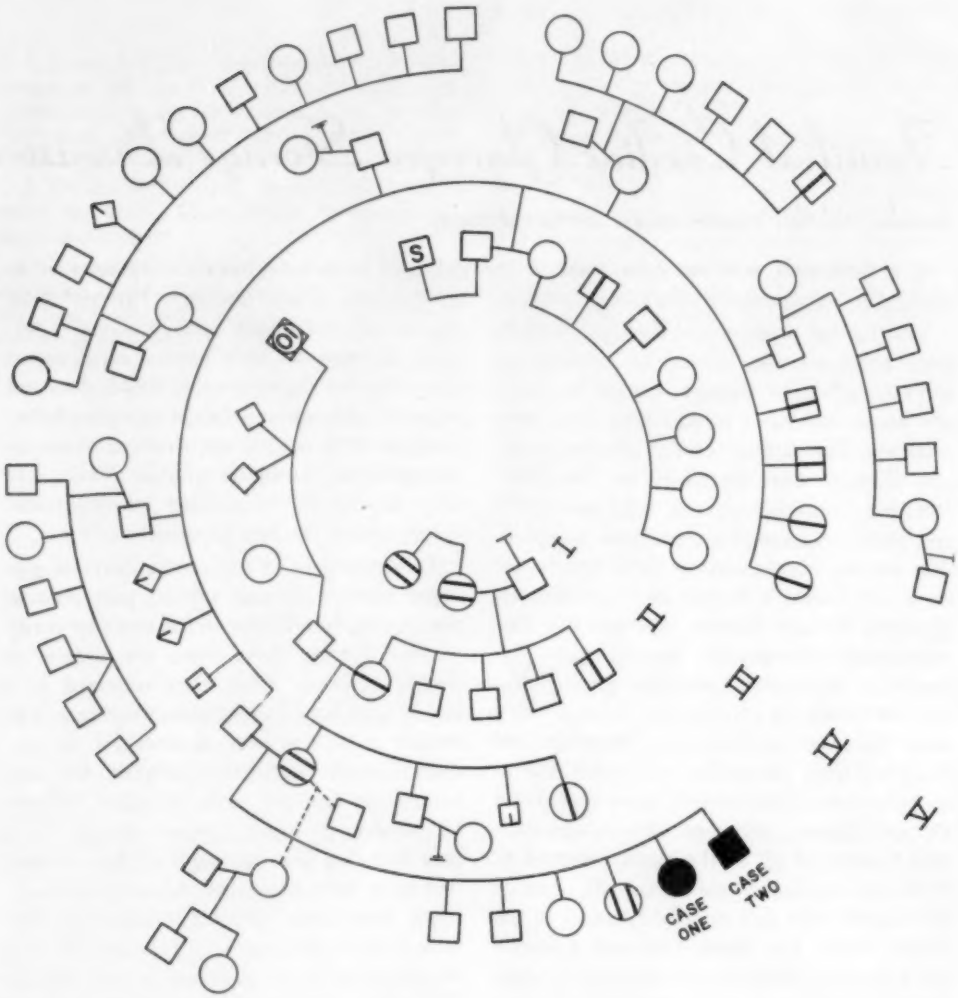
From the Department of Pathology, University of Michigan.

* References 5 through 19.

† References 20 and 21.

‡ References 22 and 23.



KEY FOR SYMBOLS

PROVED GLYCOGEN STORAGE DISEASE

PROBABLE GLYCOGEN STORAGE DISEASE

POSSIBLE GLYCOGEN STORAGE DISEASE

DIABETES MELLITUS AND GIRDLE OBESITY

PROBABLE DIABETES MELLITUS AND GIRDLE OBESITY

SEX UNKNOWN MISCARRIAGE

TEN SIBLINGS S SEVERAL BROTHERS

NO ISSUE

Fig. 1.—Familial cardiac glycogen storage disease, diabetes mellitus, and girdle obesity.

included in the present report. Glucagon, which has been crystallized in pure form,[25] has been shown to be a potent hyperglycemic glycogenolytic factor. Whether or not it is produced by alpha cells has been the subject of much discussion.§ Many investigators have suggested that a deficiency of glucagon or of alpha cells might be a causative factor of glycogen storage disease.

REPORT OF CASES

Because the two patients forming the basis for this report were siblings, a thorough search was made into five generations of both the maternal and paternal lines for other cases of glycogen storage disease and also of other metabolic diseases.|| Information was secured with difficulty, particularly for the paternal line. The father of the affected siblings was chronically alcoholic and uncooperative. His health status was not investigated, although vague suggestions of ill health were offered by his wife.

Figure 1 is a diagram of this family. Of interest was the strong incidence in the maternal line of heavily muscled females with marked girdle adiposity and with diabetes mellitus, hypertension, and "dropsy." The assumption was that the mother, maternal grandmother, great-grandmother, and the great-grandmother's sister had Kimmelstiel-Wilson's syndrome. Possible cardiac glycogen storage disease is indicated by the appropriate symbol for the apparently normal children who died suddenly and unexpectedly before the age of 1½ years, a striking feature of cardiac glycogen storage disease. This had occurred in collateral lines on both sides of the family, which suggests a recessive trait. Necropsies were not performed, and these deaths may have been due to more than one cause.

History of the Mother.¶—Mrs. H. C. was 40 years old when her fifth child was born. She had had seven pregnancies. For an indefinite period she had had obesity and high blood pressure, with severe headache and dizziness. Melioration of these symptoms occurred during each pregnancy, but they again became severe after delivery. Four children, daughters of 14 and 21 years and sons of 16 and 17 years of age, were living and apparently well. The older girl was conceived in an illegitimate but nonconsanguineous mating.

Nine years elapsed after the birth of the youngest surviving child before another pregnancy occurred. During this pregnancy the mother noted relief of symptoms, with a feeling of well-being. She was obese. Blood pressure was 210/100 mm. Hg. Glycosuria, graded 2+, was found. Fasting blood glucose level or glucose tolerance was not determined. A stillborn female infant was delivered spontaneously at term, and death was estimated to have occurred from one to two weeks prior to delivery. Permission for necropsy was refused. A suspicion of glycogen storage disease is now justified in view of the high incidence of this disease in siblings and also its occurrence in stillborn infants.

Two years later the sixth pregnancy ensued, again with relief of symptoms. The mother's weight before pregnancy was 250 lb. (113.4 kg.), and this increased to 277 lb. (125.6 kg.) at the eighth month of pregnancy. Her height was 5 ft. 2 in. (157.48 cm.). Blood pressure was 154/72 mm. Hg. The urine was negative for albumin and glucose. Fasting blood glucose determinations ranged between 119 and 150 mg. per 100 cc. She received desiccated thyroid gland during pregnancy. She "felt life" until just before delivery, but no fetal activity was noted by the physician. Roentgenograms revealed a breech presentation (sacrum right anterior). Delivery was uncomplicated except for the breech presentation. The infant (Case 1) was dead when delivered, with no apparent cause for a stillbirth.

The mother appeared for medical attention about one year later, complaining of a tumor in the breast. At this time she was not pregnant. Examination of the urine revealed glycosuria graded 4+. A fasting blood sugar test showed 304 mg. per 100 cc. of glucose. She was placed on a 1200-calorie diet and given insulin. Insulin dosage was stabilized at 40 units of isophane (NPH #) insulin. The breast tumor was removed and was diagnosed as "cystic disease." Symptoms improved, insulin was discontinued, but the low-calorie diet was maintained.

About one year later the seventh pregnancy occurred. The mother's weight now varied from 240 lb. (108.8 kg.) to 254 lb. (115.2 kg.) on a low-calorie, salt-poor diet. Her fasting blood sugar decreased progressively from 163 mg. per 100 cc. after two months' gestation to 123 mg. per 100 cc. (80 to 120 mg. per 100 cc. considered normal) just before term, suggesting increasing function of the

§ References 26 and 27.

|| The Department of Human Heredity, University of Michigan, and Mrs. V. S. Anderson, field investigator, supplied many of the details concerning the family.

¶ Dr. S. Philip Grillo, Belleville, Mich., and Beyer Hospital, Ypsilanti, supplied part of the clinical information.

Neutral protamine Hagerdorn.

fetal pancreatic islets. No insulin was given. Urine examinations revealed sugar (1+) at three months' gestation but none during the remainder of the pregnancy. Blood pressures varied from 150/80 to 184/104 mm. Hg. Her heart was considered normal. Delivery at term was complicated by transverse arrest necessitating the aid of midforceps. The infant (Case 2) was cyanotic and flaccid on delivery, but recovery was rapid for both child and mother.

Several months later the mother was in fair health. Her constitutional diathesis was like that of her mother and grandmother. Girdle adiposity was striking. Musculature appeared to be unusually well developed. Her face was somewhat rounded and florid. She was not taking insulin, but she was on a low-calorie, salt-poor diet. Laboratory studies were not done at this time.

Siblings of Cases 1 and 2.—The half-sister and the other three living siblings were in good health. There was no trace of glycogen storage disease or of any other metabolic disorder, although examination was directed especially toward such abnormalities. The maternal constitutional diathesis, as yet, had not become manifest in the daughters. The half-sister had married, and she had had a son and a daughter, both of whom were beyond 1½ years of age. Neither has shown evidence of the abnormalities under consideration.

Fig. 2 (Case 2).—Roentgenogram showing cardiomegaly.

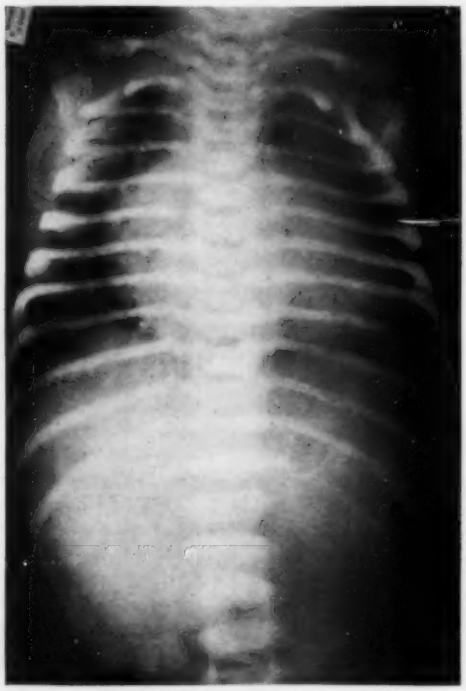


Fig. 3 (Case 2).—Diffuse cardiac and hepatic enlargement.

CASE 1.—Because the infant was stillborn, no clinical data were available.

CASE 2.—The patient was a large male infant who weighed 10 lb. (4.5 kg.) at birth. For the first five days he was sluggish and nursed poorly but otherwise was considered normal. On the evening of the fifth day he had gross hematuria, and on the sixth day he was admitted to the hospital in extremis. Physical examination showed a well-developed, large infant with cyanosis, mottled skin, dyspnea, lethargy, and flaccidity. Temperature was 101.3 F; respirations, 96 per minute, rapid and shallow; pulse, weak and 152 per minute. There was obvious cardiomegaly, but no murmurs were heard. The lungs were clear. The liver edge was 4 to 5 fingerbreadths below the right costal margin. The spleen was palpable. A chest roentgenogram (Fig. 2) confirmed cardiomegaly. An electrocardiogram showed the normal right axis deviation. Urine examination revealed no ketone bodies, but albumin (3+) was present. Fasting blood sugar levels were 101 and 105 mg. per 100 cc. An epinephrine tolerance test showed no increase in blood glucose, but the test was performed imperfectly and was not repeated. Examination of the blood showed 16.7 gm. of hemoglobin per 100 cc., with a red blood cell count of 4,670,000 per cubic millimeter. White blood cells were 11,000 per cubic millimeter, with 59% polymorphonuclear neutrophiles, 23% lymphocytes, 11% monocytes, 5%

eosinophiles, and no basophiles. Carbon-dioxide-combining power was 17 mEq. per liter, with a pH of 7.31. Total basicity was 150 mEq. per liter. Total serum protein was 5.74 gm. per 100 cc.

The patient's condition improved somewhat in an incubator with oxygen administration. He was given a transfusion of 50 cc. of whole blood and also streptomycin and penicillin. The temperature fell to 98 F. He was thought to be improving but became cyanotic whenever oxygen therapy was discontinued. On the sixth hospital day he suddenly became flaccid, dyspneic, and cyanotic, and the pulse became weak and increased to 164 per minute. Death occurred shortly thereafter, despite the administration of digitoxin and epinephrine.

NECROPSY FINDINGS IN CASES 1 AND 2

The body of Case 1 was examined at necropsy* six and one-half hours following stillbirth. Case 2 was examined 1 hour and 40 minutes after death. Although Case 1 was stillborn, death was assumed to have occurred shortly before delivery because of rigor and the absence of putrefaction. In each case the primary cause of death was cardiovascular failure. Both infants were well developed and well nourished. The marked cardiomegaly and hepatomegaly of Case 2 are illustrated in Figure 3. Other outstanding features are presented in outline in Table 1.

The adrenal glands were of interest. In Case 1 the combined weight of the adrenal glands was 18 gm. There were cortical hyper-

* Dr. Henry Bryant, Ann Arbor, Mich., performed the necropsy of Case 1 and supplied the tissues for additional studies.

TABLE 1.—*Summary of Necropsy Findings in Cases 1 and 2*

Organs	Wt., Gm. (Normal Wt.[28])	Size, Cm.	Gross Findings	Microscopic Findings	Glycogen Content (Assay and Microscopic)	Stable Glycogen (Assay and Microscopic)
Heart						
Case 1	46 (17)	6.5×5×3.5 Lt. vent. wall, 1.0 Rt. vent. wall, 0.8	Diffuse hypertrophy; subepicardial petechiae	Lace-like appearance of myocardium due to central vacuolation of muscle fibers (glycogen)	++++	+++
Case 2	61 (19)	8×6×5.0 Lt. vent. wall, 0.65 Rt. vent. wall, 0.7	Diffuse hypertrophy and dilatation; subepicardial petechiae; thrombus in lt. auricle	Similar to heart of Case 1	++++	+++
Liver						
Case 1	256 (78)	12.5×8.5×4	Hepatomegaly; congestion; yellow discoloration	Acute passive congestion; marked vacuolar change in hepatic cord cells due to lipidosis and glycogen storage; persistent glycogen in small amount	+++	+−
Case 2	172 (123)	12×7×4	Hepatomegaly; mottled red and yellow; foci of infarction from embolus from heart	Intense congestion; marked vacuolation of hepatic cord cells due to lipidosis and glycogen storage; multiple infarcts	++	−
Kidneys						
Case 1	Lt., 11	4.5×2.5×1	Fetal lobulation; congestion	Congestion and edema; vacuolar change in epithelium of loops of Henle (glycogen?)	+	−
	Rt., 13	5×3×1.5	Fetal lobulation; congestion			
Case 2	Lt., 17.8	4.5×2.5×2	80% destroyed by anemic infaret; nephromegaly; congestion	Organizing emboli and infaret of lt. kidney; glycogen deposition in loops of Henle; cloudy swelling and degenerative fatty infiltration	+	−
	Rt., 24.6	5×3.5×2	Nephromegaly; congestion			
Voluntary Muscle						
Case 1	.....	.....	Congestion	Moderate glycogen storage	+	−
Case 2	.....	.....	Congestion	Moderate glycogen storage	+	−
Pancreas						
Case 1	3.0	6×0.6×0.5	Normal lobulation	Marked hyperplasia and hypertrophy of islet cells; no stainable alpha cells; 80% to 90% were beta cells	None	−
Case 2	7.8	6×2×1.0	Normal lobulation	Marked hyperplasia and hypertrophy of islets of Langerhans; marked decrease in alpha cells with preponderance of beta cells	None	−

plasia and hypertrophy, with lipids confined to the inner one-half of the cortex. In Case 2 the adrenal glands were also enlarged, but the right was practically destroyed by infarction. The anterior lobe of the pituitary gland was considered normal in each infant.

Microscopic examination of the various organs was made, hematoxylin and eosin being used for the routine stains; Sudan III for lipids; Best's carmine and periodic acid-leucofuchsin for glycogen (controlled by diastase digestion), and Gomori's chromium hematoxylin and phloxine,[29] Gomori's rapid trichrome stain,[30] Gomori's aldehyde-fuchsin stain,[31] modifications of Masson's trichrome stain, and modifications of Gomori's stains [32] for differentiation of alpha, beta, and delta cells of islets of Langerhans.

Glycogen Content of Tissues.—Tissues from Case 1 had been fixed in 10% aqueous formalin and in absolute alcohol at the time they were received, and so special quantitative and qualitative methods could not be done. Abundant particulate glycogen was present diffusely in the myocardium and liver with traces in the loops of Henle, as shown by Best's carmine and periodic acid-Schiff's stains after both types of fixation. By accident, additional blocks from Case 1, stored in unbuffered 10% aqueous formalin, were misplaced and were subjected to alternate freezing, thawing, and relatively high temperatures for six months (sufficient to destroy glycogen in normal tissues). The excessive glycogen was still present in the heart but was absent elsewhere except for questionable traces in the liver.

For Case 2, quantitative determinations of stored glycogen were made,† using fresh samples of heart, liver, and voluntary muscle which were placed immediately in 40% potassium hydroxide. Similar fresh samples were analyzed from a control case having cardiomegaly and cardiovascular failure because of morphologic congenital heart disease. Comparisons of the amounts of glycogen are shown in Table 2.

† By Dr. Raymond L. Garner, Associate Professor of Biological Chemistry, University of Michigan.

Digestion experiments were performed which were similar to the method used by Bangle.[17] Mashes were prepared from fresh heart, liver, kidney, pancreas, and voluntary muscle from Case 2 and from a control. The mashes were added to Lillie's phosphate buffer. Microscopic sections of these tissues were incubated with the various mash preparations at 37 C for one hour. Additional microscopic tissue sections from the same organs were incubated with 1.0% malt diastase dissolved in Lillie's phosphate buffer. The tissue sections were stained with periodic acid-leucofuchsin. Stainable glycogen disappeared from the tissue sections with one exception. Stainable glycogen persisted in the heart from Case 2 when incubated with the buffered mixture containing mash from the same heart, but the glycogen disappeared when other mash preparations were used. This suggests some defect in the enzymes within the heart in Case 2.

TABLE 2.—*Relative Amounts of Glycogen by Weight in Heart and Liver of Case 2 as Compared with a Control*

Case	Heart (Wet Tissue), Per Cent	Heart (Corrected for Water Content), Per Cent	Liver (Wet Tissue), Per Cent	Liver (Corrected for Water Content), Per Cent	Voluntary Muscle (Wet Tissue), Per Cent
2	2.1	16.4	5.0	26.75	1.0
Control	0.4	1.7	1.7	6.95	...
Increase in glycogen storage disease		9.6 times		3.8 times	

A further test for the stability of cardiac glycogen in Case 2 was performed. Unfixed tissues were stored at 5 C for 30 and 60 days. Particulate stainable glycogen disappeared from all tissues tested except from the myocardium, which still showed glycogen in significant quantities after 60 days.

Stains for glycogen included Best's carmine and periodic acid-leucofuchsin stains after fixation with 10% aqueous formalin, alcohol-formalin, Bouin's solution, and absolute alcohol. Each of the aqueous fixatives preserved glycogen as well as or better than absolute alcohol.

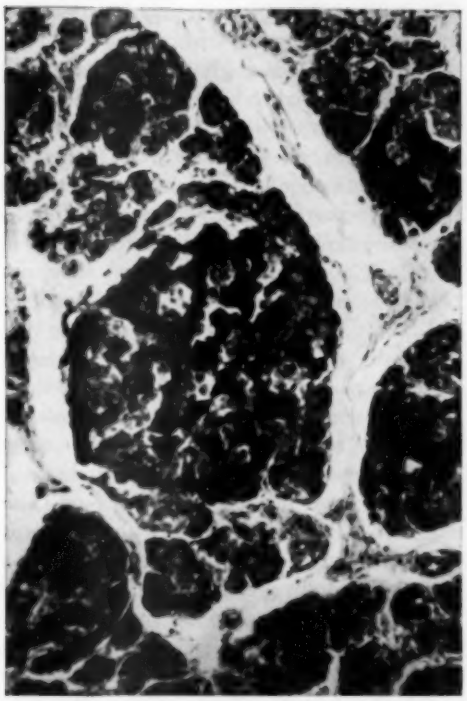


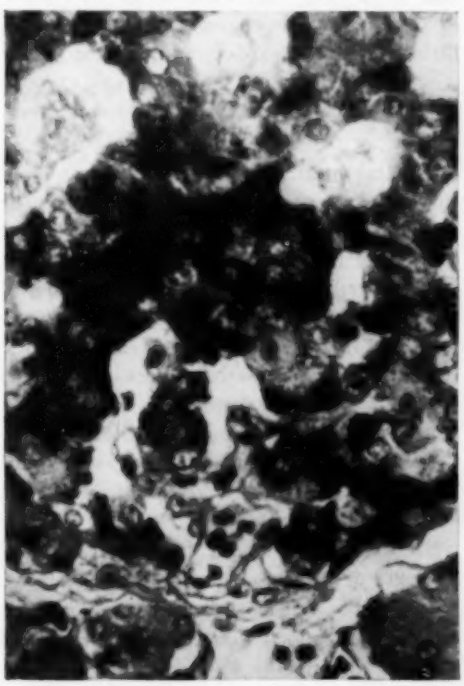
Fig. 4 (Case 1).—Abundant islet tissue with both hyperplastic and hypertrophic beta cells. Gomori's chromium hematoxylin and phloxine stains; reduced about ⅔ from mag. × 230.

MICROSCOPIC FINDINGS IN ISLETS OF LANGERHANS IN CASES 1 AND 2

It was estimated that a striking hyperplasia and hypertrophy of islets of Langerhans (Fig. 4) accounted for from 60% to 75% of the total volume of pancreatic tissue in multiple blocks representing the entire organ. Such hyperplasia is of questionable significance in view of the active neogenesis of islet tissue in the newborn. However, the islets were unusually large and the cells appeared mature, resembling those of adult pancreas. Individual islet cells were larger than those of normal infants by 50% to 100%. Of the many special granule stains used, Gomori's chromium hematoxylin and phloxine method was most useful, staining beta granules blue, and alpha and delta cells red or pink. Masson's trichrome stains differentiated alpha, delta, and beta cells. The preponderant beta cells made up from 80% to 90% of the hyperplastic and hypertrophic islet cells (Fig. 5). (Normal for infants is considered to be 30% to 50% of islet cells.) These beta cells were packed with coarse beta granules (Fig. 5), whereas normal infantile beta cells usually show only marginal granulation. Alpha cells, reputedly the source of a glycogenolytic substance ‡ known as glucagon, were absent in Case 1 and only sparsely present in Case 2. Delta cells were greatly increased and made up the remainder. (Usually delta cells are not found in appreciable numbers in the infant pancreas.) The absence of alpha cells in the newborn has been described in rats § but not in humans, although it has been suggested.[20] However, the full development of beta cells is much delayed in the rat (just prior to birth) as compared with the human (first trimester).‖ There is no reason to believe that the appearance of alpha cells in humans would coincide with that in the rat, when there is

‡ References 22 and 23.
§ References 33 and 34.
‖ References 33 and 34.

Fig. 5 (Case 1).—Heavy beta granulation, sometimes obscuring cell detail. Alpha cells were not found. Gomori's chromium hematoxylin and phloxine stains; mag. × 590.



such a discrepancy in the development of beta cells. In my experience, and according to Ferner,[35] alpha cells have made up a relatively greater percentage of islet tissue in premature and term infants than in adults. In the adult, the average range is from 5% to 30% alpha cells, 70% to 90% beta cells, and 0% to 5% delta cells.

COMMENT

From the evidence presented there can be no doubt that these cases belong in the complex of diseases known as glycogen storage disease. The massive amounts of stable glycogen which resisted glycogenolysis in the diffusely hypertrophied myocardial fibers and the early deaths from cardiovascular failure pointed conclusively to the cardiac variety of glycogen storage disease. Extensive glycogen infiltration of the heart is common in infancy and in diabetes mellitus.[36] Therefore, the mere presence of large amounts of demonstrable glycogen in enlarged organs is insufficient for the diagnosis of glycogen storage disease.[36] There must, in addition, be proof of unusual stability of that glycogen. The location of the stable glycogen and the clinical findings serve to differentiate the cardiac and hepatic forms. The numerous excellent reviews make a detailed discussion of the literature unnecessary.

Several unusual features were encountered in the family under investigation. First, the coexistence of familial diabetes mellitus and of cardiac glycogen storage disease has not been described previously, although such a relationship has been emphasized for the hepatic form of von Gierke's disease proper.[16] Much importance has been attributed to this apparent difference in the two forms of glycogen storage disease. More thorough investigation, including periodic clinical examinations of the members of families in which cases of cardiac glycogen storage disease were discovered, might have revealed the presence of mild subclinical diabetes mellitus. The functional islets of a fetus may compensate for and obscure a mild maternal diabetes mellitus, as was true in this family. The relationship between diabetes mellitus and glycogen storage disease has not been determined. Cardiac glycogen storage disease has appeared to be inherited as a simple Mendelian recessive with variable penetrance. If it proves to be related to hereditary diabetes mellitus and to other metabolic defects, the hereditary pattern may not be as simple as originally supposed. Also, as has been shown, cardiac glycogen storage disease may not always be sex-linked, as has been hypothesized.[16]

Second, the unusual features of the islets of Langerhans may have been related to maternal diabetes mellitus. The hyperplastic and hypertrophic islet tissue of adult type, with a preponderance of heavily granulated beta cells and increased numbers of delta cells, was fully compatible with the great demand upon islet tissue to compensate for maternal insulin deficiency. However, the complete absence or extreme scarcity of alpha cells has not been described, nor have I seen it in cases of maternal diabetes mellitus. In pregnant rats with alloxan diabetes the offspring show earlier development and also an increased number of alpha cells.[33] The significance of this apparent difference could not be determined. I have not found another example of hyperplasia of islet tissue in which alpha cells did not share to an equal or greater degree with beta cells in the increase of cells. McQuarrie, Bell, and associates[37] have discovered a deficiency or absence of alpha cells in two cases of familial spontaneous hypoglycemosis. These cases would tend to support the hypothesis of a deficiency of glycogenolytic substance produced by alpha cells. Furthermore, the absence or deficiency of alpha cells and of glucagon as a possible causal factor of glycogen storage disease has been suggested by a number of observers.[26]

Obviously, there has been no adequate explanation for all cases of glycogen storage disease. Most authors have felt that it is due to some deficiency of enzymes or hormones, while others have believed that the glycogen itself is abnormal. Recently Cori and his co-workers ¶ examined glycogen from 10

¶ References 38 and 39.

patients with hepatic glycogen storage disease and found the normal branching chain structure of glycogen in all except two cases. In these two cases the chain was short with less branching. In some of the remaining cases, and particularly the severe ones, there was a deficiency or absence of glucose-6-phosphatase in the liver. Apparently glucagon, the crystalline hyperglycemic factor from the pancreas,[25] influences phosphorylase activity. Therefore, an absence of glucagon might well be an important factor in the hepatic form of glycogen storage disease. However, McQuarrie and associates,# Pincus and Rutman,[26] and others have used glucagon for hepatic glycogen storage disease without improvement as measured by blood sugar levels. Corticotropin (ACTH) has been used with some benefit.[41] In spite of the evidence for the production of glucagon by alpha cells, no one has proved yet beyond doubt that glucagon is produced in the course of the normal functioning of alpha cells. There have been extensive arguments for and against this hypothesis.* Since phosphorylase is not found in cardiac muscle, there must be another defect to explain the cardiac form of the disease. The Coris and their associates have postulated a deficiency of debranching enzymes such as amylo-1,6-glucosidase for the cardiac type and also for the hepatic types in which there is an abnormal structure of the glycogen.

In the study of future cases of the glycogen storage syndrome, it is hoped that thorough enzymatic studies will be done. Also, complete studies of pancreatic islets will be needed to determine whether there is any correlation between possible enzyme deficiencies and alpha cells.

SUMMARY

Two proved cases and a possible third case of the rare familial cardiac glycogen storage disease form the basis of this study. Genealogic investigation of five generations of the family concerned reveals a high incidence of sudden, unexplained infant deaths and of hereditary diabetes mellitus with girdle adiposity. The mode of inheritance apparently is that of a non-sex-linked recessive trait. Deficiency of alpha cells must be considered as a possible causative factor in glycogen storage disease.

The relationship of this deficiency to enzymatic defects has been presented as a possible factor in the cause of the glycogen storage syndrome.

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Statistical Analysis of the Epicardial Fat Weight in Human Hearts

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Traditionally, the myocardial mass has been looked upon as the anatomic substrate of cardiac work. In turn, the total heart weight as conventionally determined at autopsy has been accepted as the gauge by which to estimate the myocardial mass. This practice implies that nonmyocardial components of the heart such as the coronary vessels, the valves, the roots of the large arteries, and the epicardial fat constitute a uniform and relatively minor source of error.

The present study intends to evaluate the influence of the epicardial fat upon the total heart weight. To this end, the weight of the epicardial fat is presented in relation to total heart weight, body obesity, sex, and age.

MATERIALS AND METHODS

In a consecutive autopsy series the hearts of all adults 21 years of age and over were analyzed. There were 75 male hearts weighing between 251 and 700 gm. and 62 female hearts weighing between 151 and 500 gm. In order to obtain at least two hearts in each weight class, five specimens scattered outside these weight limits were disregarded.

The 137 hearts were prepared by the Schlesinger technique [1] modified so as to avoid any submersion in fluids, as follows: (*a*) injection of the coronary arteries with a radiopaque gelatin mass, a heart "taking" about 10 cc. of mass; (*b*) "unrolling" of the heart, transforming the closed organ cone into a flat sheet of tissue, a method which allows for particularly easy identification of the borders of the heart chambers; (*c*) roentgenographic visualization of the coronary artery tree, and (*d*) dissection of the coronary arteries with removal of most of the contrast mass. Prior to separation of the chambers from each other in accordance with Müller's principles,[2] the epicardial fat was dissected off the myocardium with scissors. This technique is not without theoretical objection, inasmuch as it is virtually impossible to remove by sharp dissection every bit of fatty tissue from the underlying myocardium. Müller [2] found that the amount of remaining fat extractable chemically from the heart is fairly constant and does not exceed 8.1% of the weight of the dissected fat. Any bias of statistical analysis is minimized by the uniformity of the procedure.

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RESULTS

The age distribution of the 137 subjects of this study is listed in Table 1.

The hearts are divided into classes according to their total weights as conventionally determined by autopsy. The mean percentages of the epicardial fat (i. e., the ratio of epicardial fat to total heart weight) range from 12.0 to 19.9 for the men and from 12.5 to 23.5 for the women (Table 2). The differences between the means of the various weight classes are statistically not significant in either sex. As a whole, the mean percentage of epicardial fat is greater for the female

TABLE 1.—*Age Distribution of 137 Subjects Twenty-One Years of Age and Over by Decades*

	Age								
	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	Total
Men	1	1	10	9	28	20	5	1	75
Women	2	4	6	9	23	15	3	0	62

than for the male hearts, the difference being significant within 10% of probability.

The percentage of epicardial fat varies greatly from heart to heart. The coefficient of variation is large in both sexes and slightly greater for women (*c. v.*=43%) than for men (*c. v.*=41%). By comparison, body length in the same population has a coefficient of variation of only 4.8% for men and 4.5% for women. This emphasizes the magnitude of variability which pertains to the percentage of epicardial fat.

In order to evaluate whether or not there is any correlation between the epicardial fat and the obesity-leanness of the body, the panniculus adiposus of the anterior abdominal wall, as recorded routinely at autopsy, was taken as a measure of the latter. While the absolute weight of the epicardial fat rises significantly in both sexes as the panniculus adiposus increases (Tables 3 and 4), the relative amount of epicardial fat shows a significant correlation only in women (Table 3).

Age is not significantly correlated with the relative amount of epicardial fat in either sex. Age is significantly correlated with the absolute weight of the epicardial fat in women, though not in men (Table 3).

TABLE 2.—*Mean Percentage of Epicardial Fat by Sex and Total Heart Weight*

Class, Total Heart Weight in Gm.	Men			Women		
	Number of Hearts	Mean Epicardial Fat, %	Standard Deviation	Number of Hearts	Mean Epicardial Fat, %	Standard Deviation
151-200	0	....	....	2	12.5	....
201-250	0	....	....	6	15.2	7.21
251-300	10	19.9	8.19	13	19.9	9.00
301-350	14	14.9	5.66	18	17.0	8.00
351-400	12	17.7	7.35	10	14.7	6.08
401-450	13	14.9	4.90	10	18.2	6.56
451-500	6	13.1	2.00	3	22.5	7.07
501-550	9	12.0	4.24	0	....	....
551-600	7	12.5	5.29	0	....	....
601-650	2	13.2	6.86	0	....	....
651-700	2			0		
Total	75	15.2	6.21	62	17.4	7.55

TABLE 3.—*Correlation of Heart Components with Thickness of Abdominal Panniculus Adiposus and with Age*

	Correlation Coefficient	
	Men *	Women *
Thickness of abdominal panniculus adiposus		
Absolute weight of epicardial fat	0.34 †	0.45 †
Relative weight of epicardial fat	0.04	0.42 †
Weight of entire myocardium	0.28 ‡	0.14
Weight of myocardium of left ventricle	0.24 §	0.13
Age		
Absolute weight of epicardial fat	0.06	0.28 ‡
Relative weight of epicardial fat	0.01	0.13

* Correlation with abdominal panniculus adiposus based on 66 men and 57 women; correlation with age based on 75 men and 62 women.
† $P < 0.01$.
‡ $P < 0.05$.
§ $P < 0.10$.

TABLE 4.—*Mean Weight of Epicardial Fat by Sex and by Thickness of Abdominal Panniculus Adiposus*

Abdominal Panniculus Adiposus, in Mm.	Men		Women	
	Number of Cases *	Mean Weight of Epicardial Fat, in Gm.	Number of Cases *	Mean Weight of Epicardial Fat, in Gm.
1 to 5	4	43	3	21
6 to 10	11	55	8	41
11 to 20	19	58	15	50
21 to 30	18	64	12	62
31 to 40	7	69	11	74
41 to 50	6	73	7	73
51 and over	1	..	1	..

* The thickness of the abdominal panniculus adiposus was available only in 66 of the 75 men of this series, and in 57 of the 62 women.

Age is not significantly correlated with the relative amount of epicardial fat in either sex. Age is significantly correlated with the absolute weight of the epicardial fat in women, though not in men (Table 3).

COMMENT

Contrary to one's expectation, our data do not show a significant decrease of the relative amount of the epicardial fat with increase of the total heart weight; i. e., the mean percentage of epicardial fat is no greater in the small hearts than in the heavy ones (Table 2). Rather, our results agree with the hypothesis that the absolute amount of epicardial fat increases proportionately with and is thus dependent on the muscle mass.

While an average of 15.2% of the total heart weight in men and 17.4% in women is epicardial fat (Table 2), individual hearts may have the most unexpected amounts of epicardial fat, from negligible to as much as one-third or more of the total heart weight (Table 5). Still larger quantities have been encountered. Müller [2] recorded a 327 gm. female heart from which 146 gm. of fat (45% of the total heart weight) could be dissected;

he also mentioned a 494 gm. male heart with 260 gm. of dissectible epicardial fat (54%). Equally impressive is a recent Cabot case of a 58-year-old woman [3]; 51% of the 570 gm. heart was dissectible epicardial fat.

The great variability of the epicardial fat weight in the various heart weight classes (Tables 2 and 5) might account, at least in part, for two related vexing situations encountered not infrequently in pathological practice. We refer to subjects with well-established hypertension who are not wasted and whose hearts are, nonetheless, "normal" within accepted weight limits; we refer also to subjects without hypertension or cardiovascular disease whose hearts, by the same standard figures of "normality," ought to be considered hypertrophied.

TABLE 5.—*Range in Percentages of Epicardial Fat by Heart Weight and by Sex*

Total Heart Weight, in Gm.	Men		Women	
	Per Cent of Total Heart Weight		Per Cent of Total Heart Weight	
	Minimum	Maximum	Minimum	Maximum
151-200	....	....	11.6	13.5
201-250	....	....	9.5	26.0
251-300	9.7	38.0	4.2	36.0
301-350	8.0	28.7	7.6	33.5
351-400	9.0	34.3	3.8	24.0
401-450	5.7	26.0	10.5	32.0
451-500	8.0	15.5	19.0	32.0
501-550	5.7	22.0	....	....
551-600	5.7	19.4	....	....
601-650	5.7	23.2	....	....
651-700				

One may rightly question the functional identity of two hearts merely on the basis of identical total weights. Tables 2 and 5 illustrate that of two male hearts each weighing 400 gm. one may have a muscle mass of 360 gm. (epicardial fat, 40 gm.) and the other a muscle mass of 260 gm. (epicardial fat, 140 gm.). It seems reasonable to interpret the difference of 100 gm. as reflecting differences of demands and work performance. It is even possible that the first heart is actually hypertrophied, although its total heart weight still falls within accepted limits of normality. By comparison, a 450 gm. heart, though prima facie hypertrophied, may, in fact, have some 20 gm. less muscle mass (epicardial fat, about 110 gm.) than the 400 gm. heart with 360 gm. of myocardium. It is obvious that in order to be truly useful any standard figures of normalcy need be complemented by an analysis of the anatomic constituents which go to make up the totality of the heart weight.

Müller,[2] in his classic monograph *Die Massenverhältnisse des menschlichen Herzens,* gave an extensive analysis of the weight relationships of the different components of the heart. Based on 867 subjects (477 men, 390 women) 21 years of age or older, he concluded, as regards the epicardial fat: (1) that there is a linear correlation between the absolute weight of the epicardial fat and the thickness of abdominal panniculus adiposus in both sexes; (2) that women have smaller amounts of epicardial fat than men with identical thickness of panniculus adiposus, and (3) that the absolute weight of the epicardial fat increases with advancing age in both sexes, except in women during their sixth decade.

Our findings are in agreement with the first and second conclusions of Müller, as listed above. In addition, we found the linear correlation between absolute weight of epicardial fat and thickness of panniculus adiposus in both sexes to be statistically significant (Table 3). As to the second conclusion, the data of Table 4 indicate that with progressive obesity the increment of epicardial fat is greater in women than in men and that, therefore, the sex difference between the absolute epicardial fat weights is more marked in lean persons than in obese ones.

The great similarity of the findings of Müller and of ourselves is all the more noteworthy because of the differences not only in ethnic composition but also in nutritional state and age between the two samples: In Müller's series, about 80% of the cases in both sexes had a panniculus adiposus of 10 mm. or less. In our series, the situation is reversed, 80% of both men and women having a panniculus adiposus 10 mm. or over (Table 4). In Müller's series, 43% of the men and 40% of the women were over 60

years of age. In our sample, the corresponding figures are 73% and 58%, respectively (Table 1).

Our data do not conform entirely with the third conclusion of Müller inasmuch as in our sample a significant though low correlation between age and absolute epicardial fat weight is demonstrable for women but not for men (Table 3). This disagreement, however, may not be valid because the difference between the two correlation coefficients in our sample is statistically not significant.

Objections to the statistical treatment of our data may be raised on the ground that the correlation between age and epicardial fat may not be rectilinear.[4] However, we were unable to discern in the scatter diagrams any other, i. e., nonlinear, correlation.

Our exposition is not to be applied immediately to the general living population, inasmuch as the sample is derived from autopsies and biased at least with respect to ethnic grouping.

The preceding statistical analysis of the epicardial fat is presented as an introduction to a larger biometrical study aimed at establishing pathological criteria of cardiac hypertrophy. A preliminary exposition of several aspects of weight of the myocardium as related to body obesity, height, and age is appended.

A significant linear correlation between panniculus adiposus and total myocardial mass was found in men, though not in women (Table 3). This correlation remains unchanged after elimination of such possibly influential factors as age and height (coefficients of linear correlation) and is not altered by eliminating from statistical analysis all hearts with valvular deformities. We presume that this correlated increase of myocardial mass with body obesity in men is largely due to hypertrophy of the left ventricle ($P<0.10$; Table 3). We suspect the lack of a relationship in women between body obesity and myocardial mass to reflect sex differences in the association between body obesity and hypertension, coronary atherosclerosis,[5] and perhaps other factors influencing myocardial weight. Because cardiac hypertrophy implies diminished cardiac reserve, the warnings by life insurance statisticians that body obesity reduces longevity seem to apply more to men than to women. In a study based on data of the Metropolitan Life Insurance Company for the period 1922 to 1936, "there was some indication . . . that overweight is relatively less harmful to women than to men in terms of its effect on longevity."[6]

Since the panniculus adiposus is significantly correlated with the weight of the myocardium only in males and the correlation between panniculus adiposus and absolute weight of the epicardial fat is significant in both sexes (Table 3), the relative amount of epicardial fat should follow the panniculus adiposus more closely in females than in males. This was found to be the case and implies that a heavy heart in an obese man is more apt to be indicative of muscular hypertrophy, whereas in an obese woman the increase in total heart weight is more likely to be indicative of epicardial obesity.

CONCLUSIONS

A study is presented of the quantitative aspects of the dissectible epicardial fat of 75 men and 62 women.

There is no significant difference in the percentage of epicardial fat between small and big hearts in the same sex. However, the percentage of epicardial fat tends to be greater in women than in men.

The variability of the percentage of epicardial fat is of considerable magnitude in both sexes; it is greater in women than in men. In the present series, as much as one-third and more of the total heart weight may consist of epicardial fat. Reasons are given for the fact that in the individual case erroneous conclusions may be drawn regarding hypertrophy versus normotrophy if the weight of the epicardial fat is not considered.

The absolute weight of the epicardial fat shows a significant linear correlation with body obesity in both sexes. However, the relative weight of the epicardial fat is so correlated only in women.

A heavy heart of an obese man is more likely to be related to an increase of myocardial mass; a heavy heart of an obese woman is more likely to reflect a larger amount of epicardial fat.

Mindel C. Sheps, M.D., M.P.H., Research Associate in Biostatistics at Harvard Medical School, read the manuscript critically and offered helpful suggestions. Marilyn Welch Monkman, B.A., rendered assistance in the collection of the data.

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Cortisone Overdosage in Rheumatoid Arthritis

Arterial and Parenchymatous Necroses; Autopsy Case Report

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Now that cortisone therapy is such a popular topic among the lay people, this case is thought to be of timely interest because it demonstrates the possible dangers of self-administered cortisone.

A 44-year-old white farmer took 300 mg. of cortisone daily for six months, without medical supervision, to treat his chronic arthritis. A duodenal ulcer developed and the patient died in a state of shock.

Postmortem studies revealed fat depletion and necrotic foci of the adrenal cortex, a duodenal ulcer, necrotizing arteritis, as well as necrosis and hemorrhage in several organs.

CLINICAL DATA

A 44-year-old white man had been suffering from rheumatoid arthritis for the past five years. Since 1950, he took cortisone intermittently with good results under physician's orders until March, 1954. His blood pressure was 120/80 at that time. He then absorbed 300 mg. of cortisone daily, for six months, without medical supervision, to relieve the pain in the joints and to do his farming as desired. Two months after the beginning of this self-administration of cortisone—five months prior to death—symptoms of duodenal ulcer without hematemesis developed. Two weeks before death the patient complained of numbness in the left foot as well as in both hands, and was told that he had a moon face. He stopped cortisone administration, and the ulcer symptoms decreased along with palliative therapy but the arthralgia flared up, accompanied by anorexia, a temperature of 104 F (40 C), perspiration, chills, hacking cough, rales in both bases, and pale foamy sputum. The patient became worse and was sent to the hospital on Oct. 3, 1954, with the diagnosis of rheumatoid arthritis, cortisone overdosage, and duodenal ulcer. On the way to the hospital a painless flaccid paralysis of the left forearm developed, extending gradually within three days to the right arm and both legs.

Upon admission, the temperature was 98.4 F (37 C), the blood pressure 100/70, the pulse rate 85. The paralytic symptoms slightly improved after potassium therapy. A total of 400 mg. of cortisone (Cortone) and 120 mg. of corticotropin was given intramuscularly during the seven-day hospital stay. The systolic blood pressure varied between 174 and 80, the diastolic between 120 and 56. Two days before death marked restlessness and generalized purpura appeared. One day before death a paralytic ileus developed, with painful abdominal distention and vomiting. Wangensteen suction was used. Twelve hours before he died, the patient went into shock. The blood pressure was 90/56 immediately before death, which occurred on Oct. 10, one week after admission.

LABORATORY DATA

Blood: Red cells, 3,400,000; hemoglobin 72%; white cells, 18,450; differential: 86% segmented, 2% band, 12% lymphocytes. Clot retraction, within one hour. Prothrombin time, 18 sec. (control 15 sec.); NPN, 78 mg. per 100 cc.

Urine: Reaction, acid. Albumin, 1+. Sugar, negative. Specific gravity, 1.018.

AUTOPSY

Autopsy was started one hour after death. External examination of the body reveals purpura, a mild cyanosis of the finger nails and toenails, a thickening of the joints between phalanges.

Thoracic Status.—Heart: The two leaflets of the pericardium are hemorrhagic and adherent to each other. The endocardium, myocardium, and coronary arteries show no change.

Lungs: Bilateral fibrous pleural adhesions and subpleural blebs are noted. The cut surface of the parenchyma shows moderate congestion and no edema. The hilar lymph nodes are small. The arteries are smooth and contain no thrombi. A small amount of mucus covers the hyperhemic bronchial mucosa.

Abdominal Status.—A total of 100 cc. of fresh blood is found in the pouch of Douglas.

Liver: The capsule is smooth. The cut surface is brown and well lobulated. No thrombi are seen in

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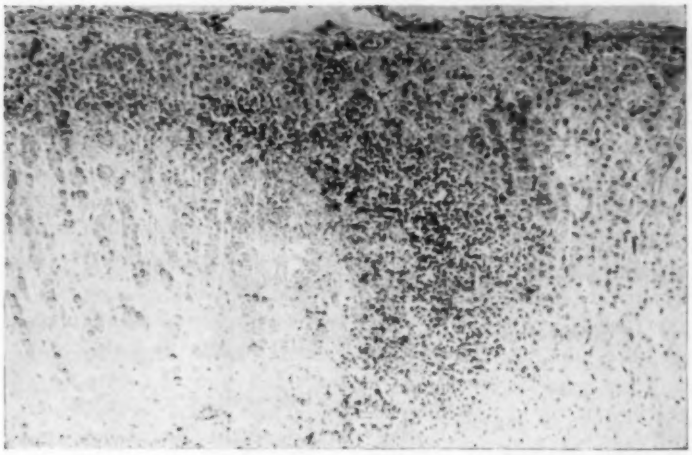

Fig. 1.—Adrenal cortex. Margin of necrotic focus. Granulocytic infiltration. Hematoxylin-eosin, low-power.

the portal vein and inferior vena cava. The gall bladder contains no stones.

Spleen: The capsule is smooth. It is bulging in one region, and the corresponding parenchyma shows a firm V-shaped area, 1 cm. in diameter, partly necrotic and partly hemorrhagic.

Stomach: It is not dilated. Its mucosa exhibits petechiae, confluent hemorrhagic foci, and erosions. An oval duodenal ulcer, 1 cm. in the greatest diameter, is found at the margin of the pylorus. Its base is pale.

Intestine: The loops are moderately distended and markedly hyperhemic. The mucosa reveals petechiae, confluent hemorrhages, and erosions.

Large Intestine: The serosa is smooth and pale. The mucosa is generally pale, but petechiae are noted in the distal segment of the sigmoid.

Pancreas: The parenchyma is well lobulated.

Adrenals: The two adrenals have the same appearance and weigh 12 gm. together. They show an autolyzed medulla and a thin pale cortex. Large dark obliterated vessels are seen in the periadrenal fat.

Kidneys: The fibrous capsule strips with ease. The capsular surface of the parenchyma is granular and hyperhemic. The cut surface shows a 0.5 cm. thick cortex and preserved striations. The mucosa of the pelvis is pale. The ureters are not dilated. A moderate hemorrhagic infiltration of the posterior abdominal wall is noted.

Pelvic Organs: The urinary bladder is not distended, and its mucosa is pale. No blood is found in the urine. The prostate is not enlarged. The tubules of the testes string out with ease.

Cranial Cavity: Not examined.

Weights and Measurements.—Heart: Weight, 355 gm. Aortic valve circumference, 7 cm.; mitral, 10 cm.; tricuspid, 12 cm.

Lungs: Right, 380 gm.; left, 320 gm.

Liver: 2300 gm., 28×24×7 cm.

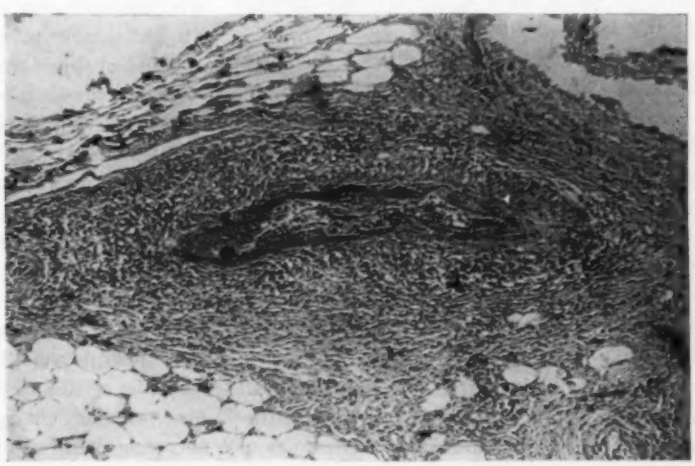

Fig. 2.—Periadrenal fat. One intact vein in right upper corner, one necrotic arteriole in the center.

Spleen: 160 gm., 10×7×3 cm.

Kidneys: Right, 160 gm., 12×7×4 cm.; left, 160 gm., 11×6×3 cm.

Adrenals (together): 12 gm.

MICROSCOPIC DESCRIPTION

Heart: Erythrocytes infiltrate the epicardium. Few granulocytes are seen at the base of the mitral valve. The blood vessels, endocardium, and myocardium show no change.

Lungs: Alternating areas of emphysema and atelectasis are noted, with no inflammatory process. Heart-failure cells are present. The wall of vessels and bronchi is not unusual. The vessels are congested. Minimal periarterial hemorrhage is seen in the hilus. The lymph nodes exhibit numerous small pale foci, some with a central granulocytic infiltration. The capillaries within the lymph nodes show no change.

Liver: The parenchyma is well preserved. The portal spaces are not infiltrated. No change is observed in the blood vessels.

Kidneys: Parietal necrosis is seen in an artery of the pelvic-medullary area, with perivascular granulocytic infiltration. Few small necrotic foci surrounded by granulocytes are noted in the medulla. Glomeruli and tubules show no pathological change.

Spleen: Several arterioles show a necrotic wall and perivascular granulocytic infiltration. An area of necrosis is seen in the red pulp, with a hemorrhagic margin infiltrated by granulocytes.

Adrenals: Some arterioles of the periadrenal fat show parietal necrosis and narrowing of the lumen. Perivascular granulocytic and mononuclear infiltration is observed. Other arterioles have a hyperplastic wall. Two cortical foci of necrosis are present, surrounded by granulocytes. The medulla shows no pathological change. Frozen sections stained with Sudan IV reveal very few small fat deposits with no zonal predilection.

Pylorus: The margin of the duodenal ulcer exhibits a necrotic surface with intramucosal sheets of granulocytes. Arterioles of the submucosa and musculosa show a necrotic swollen wall and a perivascular granulocytic-mononuclear infiltration.

Pancreas: Necrotic obliterated arterioles and foci of necrosis with granulocytes are seen.

Prostate: Some arterioles show a necrotic wall, a perivascular infiltration with granulocytes and pleomorphic mononuclears. The glands are not unusual.

Testes: Sperms are present in the tubules. Interstitial cells are scant.

FINAL PATHOLOGICAL DIAGNOSIS

History of chronic arthritis and cortisone overdosage

Necrotizing arteritis in pylorus, periadrenal fat, pancreas, kidneys, spleen, prostate

Duodenal ulcer, mucous erosions of the stomach and small intestine

Focal necroses and marked fat depletion of the adrenal cortex. Focal necroses of pancreas, kidneys, spleen, hilar pulmonary lymph nodes

Hemorrhage in skin (purpura), pericardial leaflets, gastric and intestinal wall, peritoneal and retroperitoneal space

Acute and chronic passive congestion of the lungs, minimal. Bullous emphysema, focal atelectasis, bilateral pleural fibrous adhesions

COMMENT

We felt justified in reporting this case because of the multiple lesions found in a man who had been suffering of rheumatoid arthritis for five years, and who took excessive doses of cortisone for six months, but we are unable to explain the pathogenesis of these changes step by step. The fact that cortisone produces a delay in repair makes it difficult to give an age to the cellular infiltrates observed. We have been impressed by the lack of chronicity signs in the pyloric ulcer examined microscopically, although symptoms had appeared five months prior to death. The association of arterial and parenchymatous necroses in the pylorus, pancreas, kidney, and spleen suggests—it does not prove—that the organic lesions might be related to the ischemia produced by the vascular changes. The adrenocortical necroses observed may also be attributed to

the necrotizing arteritis seen in the periadrenal fat. The veins of this adipose tissue show no change. The fat depletion noted in the adrenal cortex fits the statement of Bennett,* who showed that at least six weeks have to elapse before fat reappears after cessation of cortisone.

Cosgriff [3] reports that corticotropin and cortisone produce hypercoagulability of the blood and thromboembolic complications. Steinbrocker and co-workers [4] mention 7 cases of thrombophlebitis among 140 patients treated with cortisone and corticotropin. In our case, the prothrombin time was within normal limits, and no venous lesions were noted. We are inclined to attribute the purpura and some of the other hemorrhages observed to a pathological permeability of the capillaries induced by a potassium-sodium imbalance inherent to the adrenocortical dysfunction. This imbalance might also have been responsible for the flaccid paralysis which regressed after potassium therapy.

In conclusion, we are dealing with a case of rheumatoid arthritis treated with excessive doses of self-administered cortisone and terminating in fatal shock. Are the lesions found the result of cortisone overdosage or an association with rheumatoid arthritis? We have no criterion to answer that question. However, necrotizing arteritis has been reported in patients having received cortisone for a long period of time.† We thus believe that at least some of the parenchymatous necroses may be due to the ischemia produced by the arterial lesions. As regards the pyloric ulcer, cortisone might have had a direct action on the mucosa, or an indirect one by producing arterial necrosis.

* References 1 and 2.

† Bennett, W. A.: Personal communication.

SUMMARY

A 44-year-old white farmer had been suffering of chronic arthritis for five years and took cortisone intermittently under physician's orders with good results. He then absorbed 300 mg. of cortisone daily, for six months, without medical prescription or supervision. Symptoms of duodenal ulcer and a moon face respectively appeared five months and three weeks prior to death.

The patient was admitted to the hospital with the diagnosis of rheumatoid arthritis, cortisone overdosage, and duodenal ulcer. He was acutely ill and died in a shock-like state one week later.

Postmortem studies revealed necrotizing arteritis, focal necroses, and hemorrhage in several organs. Of particular interest are the necrotic foci of the adrenal cortex. We do not know whether these adrenal lesions are due to the arterial narrowing observed in the periadrenal fat, to the shock itself, or to the same factor which produced the necrotizing arteritis.

Dr. W. H. Pate, of Pikeville, N. C., assisted with the case history.

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Dissecting Aneurysms of the Aorta

A Study of Twelve Cases

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Improved corrective and restorative surgical procedures on the aorta have revived interest in anomalies of the large vessels and in obstructive changes and aneurysms of the aorta.* An understanding of the fundamental processes causing weakening of the wall of the aorta may be of significance in view of the possibility of taking preventive steps before actual rupture of an aneurysm occurs.[3] In the light of these new possibilities a study of dissecting aneurysms observed at necropsy at the Veterans Administration Hospital, Houston, Texas, was undertaken. The inquiry included the mechanism of their evolution and a scrutiny of the terminal clinical events.

MATERIAL AND METHODS

Among 1400 patients examined after death at this hospital between Oct. 1, 1949, and Aug. 10, 1954, twelve died with dissecting aneurysms of the aorta. The gross specimens from these patients were available for study, and, in most instances, photographs, some in color, had already been made. In each case the gross descriptions were verified by reexamination of the specimens. Blocks of tissue were obtained for microscopic study from the site of rupture and from the ascending, thoracic, and abdominal portions of the aorta. Paraffin sections were prepared from each block and stained with hematoxylin and eosin, Masson's trichrome, and Weigert's elastic tissue stains. All other organs, particularly the heart and kidneys, were included in the review.

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From the Department of Pathology, Baylor University College of Medicine, and the Veterans Administration Hospital.

* References 1 and 2.

CLINICAL AND ANATOMIC OBSERVATIONS

Incidence.—All but 7 of the 1400 patients (1021 white and 379 Negro) were male, and all were in the third to the ninth decade of life. Dissecting aneurysms of the aorta were observed in 7 of the 1016 white male subjects and in 5 of 377 Negro subjects. All 12 patients with dissecting aneurysms were male, and 9 were in the sixth decade of life; the youngest was 38 and the two oldest were 63 and 64 years, respectively.

Sites of Rupture.—The initial rent in the wall of the aorta was in its ascending portion in 10 instances and in the arch and in the abdominal aorta in 1 instance each. The dissection involved the arch in four instances, descended to the thoracic portion in two, extended the whole length of the aorta in five, and involved only the abdominal aorta in one. Proximal intimal defects were identified in 10 instances, and distal defects, in 3. Hemorrhage beyond the vessel wall into serous cavities occurred in seven instances; into the pericardial cavity in five and into the left pleural cavity in two.

Changes in the Aorta.—The principal changes in the aorta were the tear in the intima at the site of rupture in the wall and the split usually in the media, with the accompanying hemorrhage into the adventitia and adjacent structures. These changes are well illustrated in Figures 1, 3, 5, and 7. The principal spread of extravasation of blood usually occurred distally from the site of rupture. In most instances there was also a proximal spread of the extravasation. In either direction the hemorrhage was in the media or between the media and the adventitia. In no instance was there an aneurysmal dilatation of the aorta, nor were any excessive gross atheromatous or sclerotic changes observed. In fact, in 10 of the 12 pa-

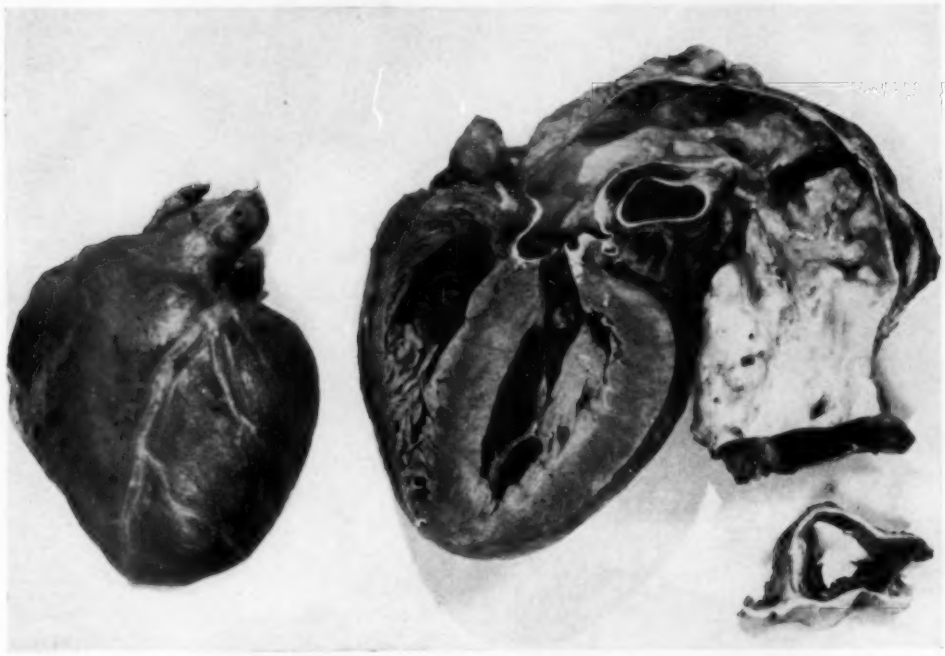

Figure 1

Figs. 1 and 2.—Dissecting aneurysm of the aorta in a white man aged 58 years (A-4). The intimal tear is 3 cm. distal to the orifice of the left subclavian artery. The dissection extended to the diaphragm, splitting the media around most of its circumference. The intima is broadened by atheromatous change. The elastic fibers of the media are split near the outer margin, and clotted blood replaces the adventitia. Weigert preparation; reduced slightly from mag. × 20.

tients the initial rent was in the ascending portion of the aorta, where atheromatous and sclerotic changes are usually least marked.

The pertinent microscopic appearances are illustrated in Figures 2, 4, 6, and 8. The wall of the aorta was weakened, and the elastic fibers were interrupted at the site of rupture. The "give" may have been at any point in the media, close to the intima or to the adventitia. Although atheromatous change was occasionally observed near the site of hemorrhage, it appeared to bear no direct relation to it. The defect in the wall of the aorta was in the media and apparently independent of any discernible previous change. The media appeared to be split by extravasated blood. On cross section the space in the media filled with blood had the shape of a crescent.

Figure 2

Changes in the Heart and Kidneys.—In all instances the weight of each kidney was 50 to 100 gm. less than usual, with microscopic evidence of nephrosclerosis. In all instances the heart was 50 to 500 gm. heavier than

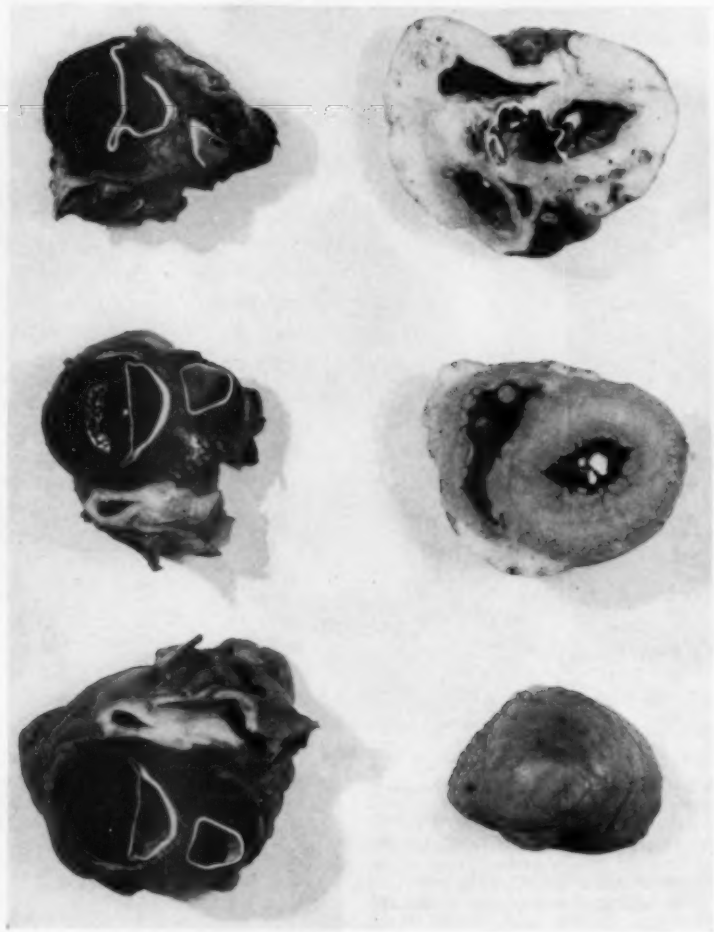

Figure 3

Figure 4

Figs. 3 and 4.—Dissecting aneurysm of the aorta in a white man aged 56 years (A-7). There is a split in the outer media filled with clotted blood. There is a rupture of the adventitia 2 cm. above the aortic orifice. Clotted blood surrounds the roots of the aorta and pulmonary artery. Blood filled the pericardial cavity. Through the tear in the intima and media clotted blood protrudes. The remaining portions of the media are separated by clotted blood. Weigert preparation; reduced about ¼ from mag. × 20.

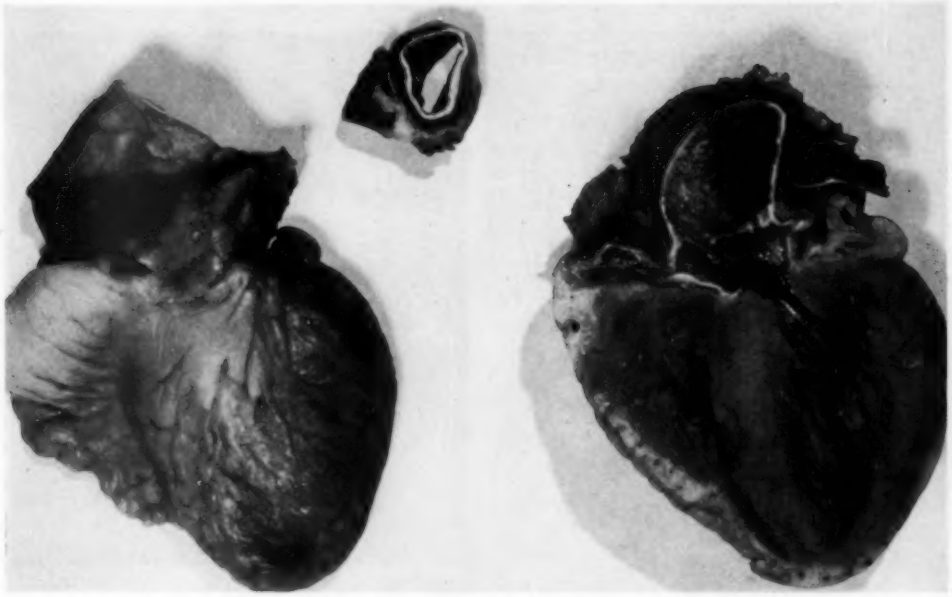

Figure 5

Figure 6

Figs. 5 and 6.—Dissecting aneurysm of the aorta in a white man aged 56 years (A-8). There is a split in the media up to 2 cm. wide filled with clotted blood. The split involves over half of the circumference of the aorta from 1 cm. above the aortic orifice almost to the innominate artery. There was a tear in the adventitia 1 cm. below the intimal defect, and extravasated blood filled the pericardial cavity. There are atheromatous changes in the intima and media, with separation and interruption of the elastic fibers. Clotted blood is seen along the outer margin of the media, separating it from the adventitia. Trichrome preparation; × 20.

usual, with microscopic evidence of cardiac hypertrophy and scarring of the myocardium. Hypertension was observed or inferred in all 12 patients (Table).

Terminal Illness.—Sudden severe pain in the chest, back, or abdomen was recorded in nine patients. The duration of terminal illness was from 1 to 3 days in four of these patients, 8 to 11 days in three, and 30 and 105 days, respectively, in two. One patient died of encephalomalacia, one was dead on arrival, and one died six days after suprapubic prostatectomy. The clinical diagnosis of dissecting aneurysm was made or inferred in only 2 of the 12 patients.

Causes of Death.—In 9 of the 12 patients the rupture and dissection of the aorta were considered the immediate cause of death. There was massive hemorrhage into the pericardial cavity in five patients and into the left pleural cavity in two. The immediate causes of death of the other three patients were diffuse acute peritonitis from occlusion of the

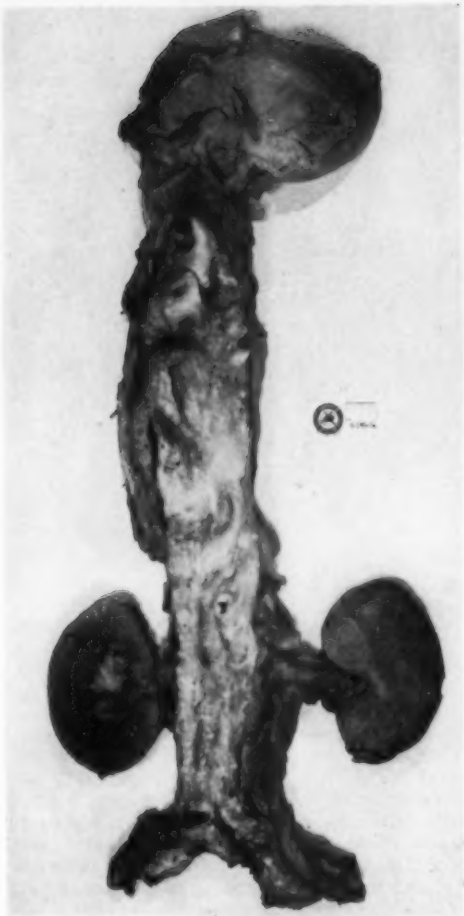

Figure 7

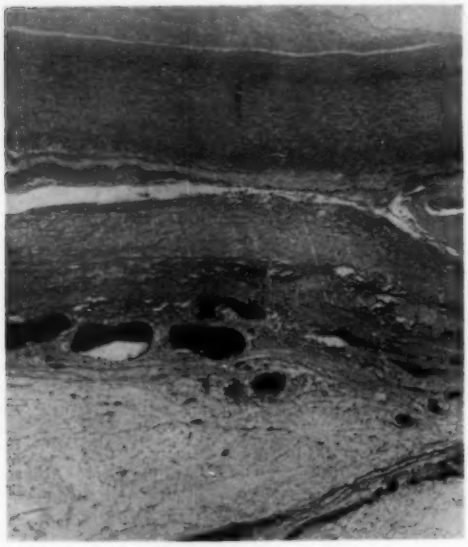

Figure 8

Figs. 7 and 8.—Dissecting aneurysm of the aorta in a Negro man aged 64 years (A-12). There was a circumferential defect in the intima of the aorta 3 cm. above the aortic orifice and a split in the media along the entire length of the aorta. There was a hematoma, 6×4 cm., that covered the tear in the adventitia of the thoracic portion of the aorta, with massive hemorrhage into the left pleural cavity. There is a slight broadening of the intima. In the outer portion of the media the elastic fibers are split and interrupted, forming a slit-like space. The lumina of the vasa vasorum are distended with erythrocytes. Weigert preparation; reduced slightly from mag. × 20.

superior mesenteric artery and gangrene of the small intestine, pontine hemorrhage and pneumonia, and encephalomalacia and focal pneumonia.

REPORT OF CASES

The following reports illustrate the clinical manifestations and the anatomic changes observed.

Case 1 (A-4).—C. B., a 58-year-old white man, was admitted Dec. 4, 1950, with a history of pain in the chest for two months and dyspnea and orthopnea for six days. Two months before admission the patient was awakened by severe pain in the abdomen, radiating to the shoulders, and dyspnea. The pain gradually diminished and became gnawing and localized at the tips of the scapulae. Six days prior to admission the pain became worse, with orthopnea, productive cough, and peripheral edema. He had been in a hospital on four occasions in 11 years because of hypertension and cardiac decompensation.

At the time of admission he was in moderate respiratory distress. The blood pressure was 220 systolic and 150 diastolic. There was dullness in the two lung bases with rales, enlargement of the heart and liver, and marked peripheral edema. There were Grade III funduscopic changes.

The urine contained albumin. A Fishberg test revealed a maximum concentration of 1.013. The blood urea nitrogen was 51 mg. per 100 cc. Roentgenograms of the chest revealed the aorta to be tortuous.

The patient continued to have pain, episodes of vertigo, and auricular fibrillation. He remained in mild to moderate congestive failure that was resistant to treatment. He was a problem in electrolyte balance. Three months after admission he developed

Patient	Age, Race, Sex	Terminal Clinical Manifestations	Duration, Days	Wt. of Organs, Gm., Blood Pressure	Changes in Aorta	Cause of Death	Other Findings
A-1 N-50-50	54 W M	Sudden low back pain spreading into abdomen; unviable ileum at laparotomy	10	Heart 500 Kidneys 400 240/125	Clotted blood in media about superior mesenteric artery and at bifurcation of aorta	Peritonitis, acute, diffuse	Nephrosclerosis; cardiac hypertrophy; gangrene of small intestine
A-2 N-54-50	58 N M	Sharp, periumbilical pain; abdominal tenderness; anasarca	30	Heart 650 Kidneys 500 160/80 rt. 130/80 lt.	Tear in intima 2 cm. above aortic orifice; medial split to left subclavian artery with thrombus in common carotid	Dissecting aneurysm of aorta	Nephrosclerosis; cardiac hypertrophy; passive hyperemia of viscera; pneumonia, focal, bilateral
A-3 N-16-51	55 N M	Precordial and left flank pain; dyspnea and restlessness; hydrothorax, ascites, distended neck veins, pedal edema	8	Heart 720 Kidneys 200 240/120	Split in media above aortic orifice to renal arteries; rupture of adventitia in thoracic portion	Dissecting aneurysm of aorta with rupture and hemothorax, left	Nephrosclerosis; cardiac hypertrophy and dilatation; passive hyperemia of viscera
A-4 N-59-51	58 W M	Pain in left chest radiating to epigastrium; peripheral edema; albuminuria	105	Heart 820 Kidneys 360 220/150	Intimal tear 3 cm. distal to subclavian artery, and one 11 cm. below; medial split partly filled with thrombi	Pneumonia; pontine hemorrhage	Nephrosclerosis; cardiac hypertrophy; passive hyperemia of viscera, anasarca
A-5 N-170-51	56 N M	Roentgen evidence of tortuosity of aorta; hemiplegia, right	35	Heart 430 Kidneys 250 190/125	Intimal tear 1 cm. above left coronary orifice; split in media to pericardial reflection	Encephalomalacia; focal pneumonia	Nephrosclerosis; cardiac hypertrophy; scarring of myocardium; hyperplasia of prostate
A-6 N-210-51	63 W M	Sharp epigastric pain, vomiting; gangrenous small intestine at laparotomy	2	Heart 490 Kidneys 360 210/115	Intimal tear 3 cm. above aortic orifice; media split to level of iliac arteries; rupture into pericardial cavity	Dissecting aneurysm with rupture into pericardial cavity	Nephrosclerosis; cardiac hypertrophy; passive hyperemia of viscera; hyperplasia of prostate
A-7 N-224-51	56 W M	Dead on arrival; became comatose and died in 30 min.	1 hr.	Heart 320 Kidneys 360	Fusiform dilatation of ascending aorta; tear in intima 2 cm. above aortic orifice; split in media to level of diaphragm; rupture into pericardial cavity	Dissecting aneurysm with rupture into pericardial cavity	Nephrosclerosis; passive hyperemia of viscera with hydrothorax, bilateral; encephalomalacia of cerebrum; hyperplasia of prostate
A-8 N-258-51	56 W M	Sudden sharp, substernal pain radiating into neck and arms to left chest and scapula; ECG evidence of myocardial damage	1	Heart 500 Kidneys 350 180/100	Vertical intimal tear 3 cm. above aortic orifice; media split to innominate artery; rupture into pericardial cavity	Dissecting aneurysm with rupture into pericardial cavity	Nephrosclerosis; cardiac hypertrophy; passive hyperemia of viscera; malacia of basal nuclei; hyperplasia of prostate
A-9 N-3-53	57 W M	Sudden severe low back pain; vomiting; restlessness and confusion; he became unresponsive, anuric, and uremic	11	Heart 470 Kidneys 340 260/175	Intimal tear 6 cm. above aortic orifice; media split to iliac arteries	Uremia; pneumonia	Nephrosclerosis; cardiac hypertrophy and dilatation; passive hyperemia of viscera with hydrothorax, bilateral; cortical hyperplasia of adrenals
A-10 N-70-53	58 W M	Crushing substernal pain	3	Heart 520 Kidneys 410 135/50	Transverse intimal tears 1 and 4 cm. above aortic orifice; split in media to renal arteries; rupture into pericardial cavity	Dissecting aneurysm with rupture into pericardial cavity	Nephrosclerosis; cardiac hypertrophy and dilatation; passive hyperemia of viscera
A-11 N-153-54	38 N M	Dyspnea, orthopnea, edema and syncope; generalized edema; severe epigastric pain	1	Heart 610 Kidneys 320 240/160	Intimal tear in arch of aorta; split in media of arch; rupture into pericardial cavity	Dissecting aneurysm with rupture into pericardial cavity	Nephrosclerosis; cardiac hypertrophy, scarring of myocardium and dilatation; passive hyperemia of viscera with hydrothorax, bilateral
A-12 N-190-54	64 N M	Episodes of acute retention; roentgenographic widening of aorta; prostatectomy for carcinoma; coma	7	Heart 450 Kidneys 300 150/100	Intimal tear above aortic orifice; media split to iliac arteries; organizing hematoma over midthoracic aorta	Dissecting aneurysm with rupture into left pleural cavity	Nephrosclerosis; cardiac hypertrophy; encephalomalacia of cerebrum and pons; residual carcinoma of prostate

right upper quadrant pain and tenderness. The stools became tarry. He soon became disoriented, lapsed into coma, and died on March 27, 1951.

At necropsy (N-59-51) the ascending aorta was 7.5 cm. in circumference and the thoracic aorta, 6.5 cm. On the convex surface of the arch, 3 cm. distal to the opening of the left subclavian artery, there was a tear in the intima, 1.5×2.5 cm., covered with a thrombus (Fig. 1). At 11 cm. below this there was a vertical tear, 1×0.3 cm. There was a split in the media of the aorta, 1 to 5 cm. wide, from the posterior portion of the arch to the level of the diaphragm. The space between the split portions of the aorta, which contained old and recent thrombi, had a smooth, gray, glistening surface. There was marked atherosclerosis of the abdominal aorta, with calcified and necrotic plaques below the renal arteries. The proximal portions of the iliac arteries were narrowed.

The pertinent findings in the anatomic diagnosis were as follows: nephrosclerosis, arterial and arteriolar; cardiac hypertrophy with scarring of myocardium and dilatation; atherosclerosis, generalized; dissecting aneurysm of thoracic portion of aorta; passive hyperemia of viscera with ascites, bilateral hydrothorax, and hydropericardium; hemorrhage into pons.

Case 2 (A-7).—E. B., a 56-year-old white man, was dead on arrival at the hospital Nov. 2, 1951. He was said to have been symptom-free, when he suddenly became unconscious and died in 30 minutes.

At necropsy (N-224-51) there was a fusiform dilatation of the ascending aorta and the proximal portion of the arch. A split in the media was filled with clotted blood, and the adventitia was ruptured 2 cm. above the aortic orifice (Fig. 3). The pericardial cavity contained 750 cc. of dark, partly clotted blood.

The pertinent findings in the anatomic diagnosis were as follows: nephrosclerosis, arterial; cardiac hypertrophy; dissecting aneurysm of ascending aorta with rupture into mediastinum and pericardium; passive hyperemia of viscera with hydrothorax, bilateral; sclerosis of cerebral arteries with encephalomalacia, focal.

Case 3 (A-8).—A. M., a 56-year-old white man, was admitted Dec. 27, 1951, with sudden severe sharp, substernal pain that radiated to the left of his neck and to both arms. The pain was first intermittent, then became constant, of less intensity, and localized to the left anterior chest and left scapula. During childhood he had had acute nephritis, and at age 24 he had had generalized edema. A diagnosis of chronic glomerulonephritis was made. Hypertension was first noted at age 48, at which time he had two cerebral vascular crises with right hemiparesis.

At the time of admission his blood pressure was 180 systolic and 100 diastolic; temperature, 101 F, and pulse rate, 80 per minute. There were cardiac enlargement and slight weakness in the right extremities.

The white blood cell count was 17,500 per cubic millimeter with neutrophiles 71%. The urea nitrogen was 12 mg. per 100 cc. There was electrocardiographic evidence of myocardial damage. Apparently the chest pain disappeared and the patient did well until 20 hours after admission, when he suddenly became cyanotic and died.

At necropsy (N-258-51) the pericardial cavity was filled with clotted blood. There was a vertical defect 1 cm. long in the intima and media on the left side of the ascending aorta, 4 cm. above the aortic orifice. There was a split in the media, up to 2 cm. wide, filled with clotted blood and involving most of the circumference of the aorta from 1 cm. above the aortic orifice to just proximal to the innominate artery (Fig. 5). There was a tear in the adventitia on the right, 1 cm. below the intimal defect. There was marked atherosclerosis of the descending and abdominal portions of the aorta.

The pertinent findings in the anatomic diagnosis were as follows: nephrosclerosis, arterial and arteriolar; cardiac hypertrophy; dissecting aneurysm of ascending aorta with rupture and hemopericardium; passive hyperemia of viscera, acute; sclerosis of cerebral arteries with encephalomalacia of basal nuclei, bilateral.

Case 4 (A-12).—J. F., a 64-year-old Negro man, was admitted July 7, 1954, with a history of "prostatism" for several years manifested by episodes of acute retention. He was otherwise symptom-free.

At the time of admission his blood pressure was 150 systolic and 100 diastolic. There were slight cardiac enlargement with an irregular rhythm and peripheral arteriosclerosis; the prostate was large and firm.

The blood urea nitrogen was 40 mg. per 100 cc.; blood sugar was 150 mg. per 100 cc., and the urine contained glucose. There was roentgenographic evidence of widening of the ascending aorta with tortuosity.

Two weeks after admission a total prostatectomy was performed for carcinoma of the prostate (S-1278-54 and S-1323-54). On the sixth day after operation he suddenly became unconscious. There were marked hypotension and respiratory distress. He remained in an unconscious state and died Aug. 6.

At necropsy (N-190-54) there was a partial circumferential defect in the intima of the aorta, 3 cm. above the aortic orifice. There was a split in the media extending the entire length of the

aorta (Fig. 7). Over the adventitia on the left of the thoracic portion was a hematoma, 6×4 cm., that covered a tear in the adventitia. The left pleural cavity contained 3000 cc. of partly clotted blood.

The pertinent findings in the anatomic diagnosis were as follows: nephrosclerosis, arterial and arteriolar; cardiac hypertrophy; dissecting aneurysm of aorta with hemothorax, left; sclerosis of cerebral arteries with encephalomalacia, focal, in cerebrum and pons.

COMMENT

Since the now classic studies of Shennan,[4] many detailed reports have dealt with the cause,† location,‡ natural history, age incidence,§ and clinical features ‖ of dissecting aneurysms of the aorta. Our data largely substantiate those of other observers. The essential nature of the lesion appears to be a weakening—"medial necrosis" of Erdheim, "faults" of Shennan—of the wall of the aorta resulting in dissection of its layers by hemorrhage. Usually there are one or more tears in the intima. There is, however, no aneurysmal dilatation in the vessel wall nor are atheromatous or syphilitic changes principal predisposing factors. The site of the initial lesion is usually between the orifice and the isthmic portion of the aorta, but, rarely, it may be in the thoracic or abdominal aorta. The sites of origin, the location, and the gross and microscopic features of so-called dissecting aneurysms of the aorta are different from local dilatations or aneurysm formations. Hence, the name "aneurysm" is not correctly applicable. The lesion consists of partial disruption of the continuity of the aortic wall by hemorrhage into the space thus formed. Hypertension appears to be the principal predisposing factor that weakens the elastic elements of the aortic wall, resulting eventually in its tearing and splitting. Alertness to the possibility of the occurrence of this lesion in any patient with hypertension might result in more frequent recognition of the lesion before death of the patient. Arteriographic examination of the aorta in instances of justifiable suspicion may permit early recognition and possible surgical correction of the lesion in an appreciable number of instances.

† References 5 through 7.

‡ References 8 and 9.

§ References 10 and 11.

‖ References 12 through 14.

SUMMARY

So-called dissecting aneurysm of the aorta was observed in 7 of 1016 white male subjects and 5 of 377 Negro male subjects examined after death. All 12 patients with dissecting aneurysm were in the sixth decade of life except the youngest, who was 38, and the 2 oldest, who were 63 and 64 years, respectively.

The initial rent in the wall of the aorta was in its ascending portion in 10 instances and in the arch and abdominal aorta in 1 instance each.

The clinical diagnosis of dissecting aneurysm was made or inferred in only 2 of the 12 patients.

Massive hemorrhage into the pericardial cavity was the immediate cause of death in five instances, and hemorrhage into the left pleural cavity, in two.

Hypertension is believed to be the most important predisposing factor in causing the weakening of the wall of the aorta that permits dissection of its layers by hemorrhage.

Because the site of origin, the location of the lesion, and the gross and microscopic features differ from those of local dilatation or aneurysm formation of the aorta, the name aneurysm is not correctly applicable.

Alertness to the possibility of occurrence of this lesion in any patient with hypertension may result in more frequent recognition during life and passible surgical correction in an appreciable number of instances.

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The Role of Brown Pigment in Experimental Hemoglobinuric Nephrosis

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During the degradation of oxyhemoglobin, methemoglobin, or myoglobin in the body, either hemosiderin or hematin may be formed. Hemosiderin has been observed in the kidneys of man * and experimental animals.† Hematin may appear in the plasma of patients after hemolysis.‡ Ferritin, closely allied chemically to hemosiderin,[7] is considered to be nephrotoxic.[8] Intravenous injection of buffered solutions of hematin into animals has proved that this substance is toxic.§ Despite the fact that either hemosiderin or hematin may be nephrotoxic, a correlation between the quantity of specific pigment per kidney and the degree of renal dysfunction has never been established. In a previous study we observed both hemosiderin and a brown pigment in the kidneys of rabbits with hemoglobinuric nephrosis. By immersing slices of fresh or formalin-fixed (one to three days) kidneys into 1% potassium ferrocyanide and 5% HCl, it was possible to distinguish between hemosiderin and a brown pigment, presumably hematin. Renal failure was more frequently associated with the retention of the brown pigment.[12] Neither the quantity nor the nature of the brown pigment, however, was established; hence the conclusions are only suggestive. It therefore seemed of sufficient importance to investigate the problem further. Accordingly, fresh kidney tissue from rabbits with hemoglobinuric nephrosis was subjected to extraction and chemical or physical analyses to determine more precisely some of the constituents.

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* References 1 and 2.

† References 3 and 4.

‡ References 5 and 6.

§ References 9, 10, and 11.

METHOD

Several factors which have been shown to accentuate the development of fatal hemoglobinuric nephrosis were employed. The rabbits were fed an oat diet supplemented with a mixture of mono- and disodium phosphate to promote an aciduria. The methemoglobin was prepared by adding 1 mg. of potassium ferricyanide for every 25 mg. of oxyhemoglobin. Each rabbit received 1 gm/kg. of methemoglobin in three or four doses in an eight-hour period.[12] Nonprotein-nitrogen (NPN) concentrations were made on autopsy serum or plasma drawn from the hearts of animals which lived five days.[13] The majority of the autopsies were performed within one hour after death. After gross examination fresh kidneys with variable quantities of brown pigment were selected for analyses. After determining the combined wet weight, one kidney was cut into 2 to 4 mm. pieces, mixed with 50 ml. of 0.3% saponin, and then homogenized in a Waring Blendor for 60 seconds. The kidney homogenate was poured into celluloid centrifuge tubes. The Blendor cup was rinsed with 25 ml. of water, and the washing was added to the homogenate. The homogenate was decanted in centrifuge tubes which were balanced and then centrifuged at 11,000 rpm for 30 minutes. The 0.3% saponin extract contains oxyhemoglobin, methemoglobin, hemosiderin, carbohydrates, and other water-soluble constituents. The extract was decanted into a graduate and the volume recorded. The hematin-like brown pigment is water-insoluble, and so it remained in the centrifuged residue. The brown pigment was extracted with 75 ml. of alkaline pyridine containing 10 parts 1 N NaOH, 28 parts pyridine

and 62 parts H_2O, as recommended by Biörck for hematin extraction.[14] The extraction of the brown pigment was insured by mechanical stirring for 30 minutes. The alkaline-pyridine mixture was poured into Stainless steel centrifuge cups. After balancing, the contents were centrifuged for 30 minutes at 11,000 rpm. The contents of the second extract were decanted into a graduate and the volume recorded. The concentrations of the water-saponin-soluble pigments and the extracted brown pigment were determined as the pyridine hemochromogen at 540 mμ in a Beckman spectrophotometer.[14] The pyridine-hemochromogen solutions were prepared by diluting 1 to 4 ml. of the different extracts to 10 ml. with alkaline pyridine. The iron concentration was determined in the water-saponin, the alkaline pyridine extract, and some kidney residues. The micromethod of Coombs was used for these determinations.[15] The fat and the renal capsule were removed from the opposite kidney. Approximately one half of the kidney was placed in a hot-air oven at 90 C to 95 C for 24 hours. From the wet and dry weight values, the per cent edema and the total dry kidney weights were calculated. It seemed desirable to resolve whether the increased dry kidney weight was due to the retention of salts or proteins. To establish this point, 50 mg. of finely powdered dried kidney was subjected to Kjeldahl digestion and analyzed for the nitrogen content. The plasma or serum NPN values are incorporated in the Table, in addition to the other analyses, so that one can observe the influence of renal injury on nitrogen retention.

RESULTS

Kidneys from 18 rabbits were subjected to air-drying or extraction and subsequent analysis. Data obtained in individual animals are shown in the Table.

The gross and microscopic kidney alterations are similar to those reported previously,[12] so that the pathologic changes will not be dealt with in this study. In 9 of 10 rabbits which died, the plasma NPN was in excess of 214 mg. per 100 cc. Death from uremia in 9 of 18 rabbits indicates the presence of severe hemoglobinuric nephrosis. A significant elevation in NPN also occurred in four rabbits (Nos. 3, 5, 6, and 7) which survived. In five other rabbits the alteration in NPN was negative, or less than 97 mg. per 100 cc. In four rabbits (Nos. 1, 2, 3, and 9) without significant renal injury, the combined wet kidney weight varied from 13.1 to 16.2 gm. In three of five rabbits (Nos. 4, 5, 6, 7, and 8) with increased wet kidney weight which survived, there was a significant elevation in plasma NPN. In 9 of 10 rabbits which died, increases in wet kidney weight varied from 20.8 to 47.8 gm. The per cent of water in kidneys with minimal involvement varied from 72.7 to 80.9. In the uremic group the

Analytical Data of Air-Dried and Extracted Fresh Kidneys

Rabbit No.	NPN, Mg. %	Autopsy, Days	Kidneys			Nitrogen in Dried Kidney, %	Saponin-Soluble Pigments, Mg./Kidney†	Alkaline Pyridine Pigment, Mg./Kidney
			Wet Wt., Gm.	Dry Wt., Gm.	Water, %			
1	24	10	13.14	3.59	72.7	....	..	0.00
2	17	10	16.17	3.60	78.5	....	..	0.30
3	116	8	13.92	2.56	80.9	12.6	23	0.00
4	82	10	27.33	3.65	86.6	....	..	1.59
5	147	11	25.03	3.70	85.2	12.9	55	2.38
6	213	11	34.33	5.01	85.4	12.2	74	4.60
7	254	11	34.72	6.56	81.1	....	..	3.12
8	55	8	25.14	3.95	84.3	....	53	3.45
9	90	4*	13.81	3.38	75.6	13.1	145	0.38
10	262	7*	27.95	5.44	80.5	....	..	11.60
11	389	5*	27.43	5.15	81.4	13.8	56	12.10
12	332	5*	24.52	4.64	81.0	14.0	83	6.10
13	356	6*	20.81	3.71	82.1	14.1	..	8.65
14	276	5*	38.07	5.50	85.6	13.6	40	10.35
15	214	8*	41.02	6.61	83.8	13.6	111	12.60
16	271	6*	31.80	5.28	83.4	12.9	75	11.45
17	286	6*	47.81	7.66	84.0	13.6	155	14.61
18	282	5*	32.27	5.64	83.1	14.7	69	14.30

* Rabbits died.

† Saponin-soluble pigments calculated as oxyhemoglobin.

values were in excess of 80.5%, indicating that edema of renal tissue is present. There were rather wide fluctuations in the calculated combined dry kidney weight. Examination of values from the normal or minimally involved kidneys shows that the dry weight did not exceed 3.95 gm. Calculated dry weights in excess of 3.95 gm. therefore show that either proteins or salts have been retained in hemoglobinuric nephrosis. The per cent of nitrogen in Kjeldahl digests did not fluctuate with variations in calculated dry kidney weight. Similar nitrogen percentages, irrespective of alterations in dry weight, suggest that the increases are due to retention of protein. It is interesting to note that in 9 of 18 kidneys the calculated dry weight was 5 gm. or more for both kidneys. In most instances the quantity of retained protein in the tubules is in excess of the quantity of methemoglobin which was injected. The increases in combined wet kidney weight are usually due to both edema and retention of protein. On occasion, however, the increased wet weight can be due principally to edema (Nos. 4 and 5). The concentration of saponin-water-soluble pyridine hemochromogen, when calculated as oxyhemoglobin, varied from 23 to 145 mg. per kidney. These fluctuations reflect, for the most part, the degree of renal congestion at the time of autopsy. There is no relationship between the retention of water-saponin-soluble pigments and the severity of the renal injury.

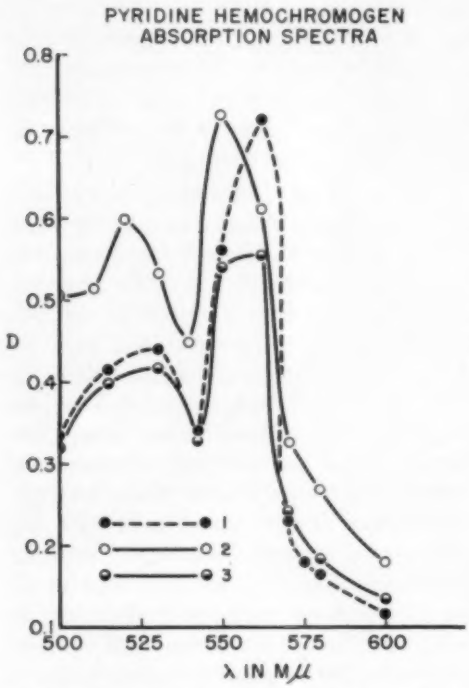
The observations made on the quantity of brown pigment extracted per kidney proved to be the most interesting. In 9 of 10 rabbits which died, there was 6.1 mg. or more of pigment per kidney. One rabbit (No. 9) which did not die of renal failure only had 0.38 mg. of brown pigment per kidney. It is also interesting to point out that animals with moderately severe nephrosis which survived also had less than 6 mg. of pigment per kidney. There is a better relationship between renal failure and the retained brown pigment than any other constituent. Since the pigment did not exceed 14.6 mg. per kidney, probably not more than 0.6 gm. of the protein precipitated in the tubules is denatured oxyhemoglobin or methemoglobin. In this extraction study, the brown pigment did not dissolve in 0.3% saponin. It has not been resolved whether the brown pigment exists in a free state or is absorbed by denatured globin or some other protein. The iron concentrations of the two extracts and the kidney residues in four animals are not shown in the Table. Minor variations in iron concentration paralleled fluctuations in the calculated oxyhemoglobin and the measured brown pigment concentrations. In no instance did we observe more than 2.5 mg. of total iron per kidney. Since the concentration of total iron did not correlate with the degree of renal failure, it seems unlikely that the tubular injury is due primarily to either hemosiderin or inorganic iron.

From 1 to 4 ml. of alkaline-pyridine extract from 10 different kidneys was diluted to 10 ml. in freshly prepared alkaline pyridine. The absorption spectrum of recrystallized hemin,‖ dissolved in alkaline pyridine at approximately similar concentration, was used for comparison in a Beckman spectrophotometer. The spectrophotometric absorption of the brown pigment extracted from kidneys was not identical to the absorption of commercial hemin. To ascertain whether freshly homogenized kidney and extraction would have any influence on commercial hemin, the following experiment was performed. A total of 20 mg. of hemin was added to fresh kidney in a Waring Blendor containing 0.3% saponin. Thereafter, the preparation was subjected to similar extraction procedures to the hemoglobinuric nephrotic kidneys. After alkaline-pyridine extraction, 85% of the added hemin was recovered. The hemin mixed with kidney and subjected to extraction had the same absorption spectrum as the original product. This indicates that the extraction procedure did not alter the hemin and suggests that alteration of the brown pigment occurs before death. Furthermore, the brown pigment recovered from different kidneys also had variations in spectrophotometric absorption.

‖ Eastman Kodak Company, Rochester, N. Y.

Three representative absorption spectra are shown in the accompanying Chart.

The greatest differences in absorption were observed between 550mμ and 560mμ. Both commercial hemin and hemin added to kidney and extracted exhibited a sharp increase in absorption from 550mμ to 560mμ (Chart, *Curve* 1). On the other hand, the absorption of brown pigment extracted from hemoglobinuric nephrotic kidneys decreased between 550mμ and 560mμ (Chart, *Curve* 2)



Alkaline-pyridine absorption spectra of hemin and brown pigment.

Curve 1, spectrum of commercial hemin added to and extracted from a normal kidney. There is a sharp increase in absorption between 550mμ and 560mμ.

Curve 2, brown pigment extracted from kidney (Rabbit 10) shows a drop in absorption between 550mμ and 560mμ.

Curve 3, pigment extracted from kidney (Rabbit 16) shows a slight rise in absorption spectrum at 560mμ.

or increased slightly (Chart, *Curve* 3). Differences in spectrophotometric absorption of the extracted pigment suggest that the brown pigment is a mixture of hematin and other degradation products of this compound. Although there is an alteration in the hematin molecule before death, the toxicity is still retained.

COMMENT

It is well known that in hemoglobinuric nephrosis there is edema with retention of proteins and pigments.[16] Quantitative correlations, however, have not been made on the relationships between the retention of these constituents and the degree of renal failure. In this study it was possible to show that the increased wet kidney weight, as might be expected, is usually due to edema and retention of proteins. It is also interesting to note that the increase in wet kidney weight may be due on occasion principally to edema. Increases in the calculated dry kidney weight suggest that at times the weight of the proteins retained in the tubules may be equal to the original kidney weight. Both the brown pigment concentration values per kidney and the quantity of methemoglobin injected per animal indicate that proteins other than denatured oxyhemoglobin or methemoglobin are retained in the tubules. Many of the brown pigment casts from fresh kidneys are not soluble in 0.3% saponin. Since oxyhemoglobin, methemoglobin, and hemosiderin are all known to be water-soluble, the water-insoluble pigment casts are probably composed of either denatured globin or plasma protein and hematin or its derivatives. Hematin plus denatured protein complexes would explain the inability to demonstrate free ferric iron in pigment casts ¶ and their insolubility in water. This observation in rabbits is in disagreement with the findings of Harrison and co-workers.[17] These workers failed to recover hematin from frozen sections of dog kidneys with hemoglobinuric nephrosis. The yellow-brown casts which were removed dissolved in buffered solution and had an absorption spectrum of methemoglobin. In previous experiments we have repeatedly observed both native and denatured methemoglobin in the urine of rabbits following methemoglobin injection. In the present study

¶ References 4 and 16.

unaltered methemoglobin would have dissolved in the 0.3% saponin solution. We believe that methemoglobin is not nephrotoxic unless it undergoes denaturation for the following reasons: After intravenous injection methemoglobin may be rapidly reduced to oxyhemoglobin without producing toxic effects,[15] and at equimolar concentrations the injection of methemoglobin into animals is not comparable to the toxicity of buffered hematin.# Even though hematin is toxic, we were not able to produce either an oliguria or a brownish discoloration of the kidneys following hematin injection.[4] We have also observed that methemoglobin may be nontoxic after injection into rabbits excreting an alkaline urine with a low phosphate concentration. Animals fed diets which promote an aciduria and increased phosphate excretion, however, develop hemoglobinuric nephrosis after methemoglobin injection.[12] Why an acid urine and elevated urinary phosphates predispose to hemoglobinuric nephrosis when combined with methemoglobin injection is not known. Nevertheless, at equimolar concentrations injection of methemoglobin into rabbits fed an acid diet is followed by the retention of much more brown pigment in the renal cortex than when hematin is employed. The fact that the brown pigment from fresh kidneys is not water-soluble suggests that the methemoglobin has undergone denaturation, probably in the renal tubules. Methemoglobin denaturation in the renal tubules would account for the retention of more brown pigment in the renal cortex. In our study of the different constituents analyzed, the best relationship was found between renal failure and the retention of brown pigment per kidney. Comparison of the spectrophotometric absorption of the pyridine hemochromogen of extracted brown pigment with commercial hemin revealed some differences. These differences in absorption are probably due to partial degradation of hematin in the body before death. The discrepancy between the findings in this study and the observations of Harrison and co-workers may be due to the fact that different time intervals and methods of analysis for hematin were employed. In view of the demonstrated toxicity of hematin, the close relationship between renal failure and the retention of brown pigment in the kidneys, and the spectrophotometric similarities between commerical hemin and the extracted brown pigment, we believe that the brown pigment is nephrotoxic.

References 4, 9, and 11.

SUMMARY

Eighteen rabbits with aciduria received 1 gm/kg. of methemoglobin intravenously. Of 18 animals, 9 died of fatal hemoglobinuric nephrosis within five to eight days after the injection of methemoglobin. Fresh kidneys from each animal were subjected to air-drying or extraction of the retained pigments. Nine rabbits which died in uremia had from 6.1 to 14.6 mg. of brown pigment per kidney. In eight rabbits which survived and one which died of malnutrition, the concentration of brown pigment varied from 0.00 to 4.60 mg. per kidney. The pyridine-hemochromogen absorption spectra of the extracted brown pigment and commercial hemin were somewhat similar but not identical. It is suggested that the difference in absorption of the brown pigment may be due to partial degradation of hematin in the body prior to death. The established relationship between the concentration of brown pigment in the kidney and the severity of the uremia suggests that this pigment is nephrotoxic.

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A Pathologic Study of Vitamin B_{12}-Deficient Chick Embryos

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Olcese and associates [1] in 1950 reported that eggs obtained from hens fed a purified synthetic-type diet deficient in vitamin B_{12} failed to hatch. The peak of mortality occurred between the 16th and the 18th day of the incubation period. The embryos were small and hemorrhagic, and their position within the egg was abnormal. The addition of animal protein factor concentrates as a crude source of vitamin B_{12} to the diet of the hens prevented the occurrence of these abnormalities. Additional macroscopic observations on the deficient embryos were reported by Ferguson and Couch.[2] It has been shown that crystalline vitamin B_{12} injected into eggs obtained from hens on a low-B_{12} diet and the parenteral injection of this vitamin into deficient hens resulted in the development of normal embryos.*

The pathologic changes observed in these vitamin B_{12}-deficient embryos are reported.

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METHODS AND MATERIALS

Vitamin B_{12}-deficient embryos were obtained from eggs produced by Single Comb White Leghorns artificially inseminated with pooled semen from New Hampshire Cockerels. Normal embryos were from eggs produced by a White Leghorn-New Hampshire cross fed a practical ration, adequate with respect to all known nutrients.

Eighty-eight 17-day-old embryos, alive when removed from the egg, were used for histologic study. Of these, 35 were from hens fed a low-B_{12} diet as previously reported [2]; 33 from hens maintained on the same deficient diet and given crystalline vitamin B_{12} either parenterally or into the egg prior to incubation, and 20 from hens fed a complete practical ration. Only a gross examination was made on 51 additional embryos from hens on the deficient diet.

The body cavities of the embryos were opened, and the entire carcass was put immediately into either Bouin's fluid or into a 4% solution of formaldehyde. Sections were subsequently removed and paraffin sections were prepared. These were stained routinely with hematoxylin and eosin. Select sections were stained by the following techniques: Mallory's fast green and aniline blue, Heidenhain, Van Gieson, Wilder's reticulum stain, Mayer's mucicarmine, periodic acid-Schiff, osmic acid, and Masson's trichrome stain. Approximately one-half of the tissues were stained by the Sudan IV technique. Serial sections were made on specific blocks.

EXPERIMENTAL

A summary of the gross pathologic changes as observed in the 139 embryos is shown

* References 2 and 3.

Incidence of Pathological Processes in 139 Embryos Obtained from Hens Maintained on a Low-B_{12} Diet—Embryos Removed from the Egg After 17 Days of Incubation

Characteristics Observed	No.	Per Cent
Edema	114	82
General body hemorrhages	104	75
Pale, enlarged, irregular-shaped heart	113	81
Hemorrhages on heart	46	33
Enlarged pericardial sac filled with fluid	9	6.5
Pale, yellowish kidney	69	49
Hemorrhages in kidney	26	18
Fatty liver with hemorrhages and necrotic areas	113	81.3
Cerebral hemorrhage	17	12

in the Table. The embryos obtained from the eggs from hens on a vitamin B_{12}-deficient ration supplemented with vitamin B_{12} did not show any variations from the normal. The embryos from hens fed the same deficient ration without a supplement of vitamin B_{12} were smaller than the normal and those receiving the supplement (Fig. 1). Eighty-two per cent of the embryos from the hens fed the low-B_{12} diet were edematous, and seventy-five per cent of these had hemorrhages into the skin (Fig. 2). Profuse bleeding occurred when the feathers were plucked. Poor feathering was characteristic of the deficient embryos; however, no histologic variations were observed in either the squamous epithelium or the skin appendages.

Fig. 2.—Seventeen-day vitamin B_{12}-deficient embryo showing extreme hemorrhage, edema, and poor feathering.

There was considerable separation of the muscle fibers by edema fluid (Fig. 3). The muscle fibers in normal embryos have a moderate amount of fat as shown by the Sudan IV preparation. The deficient embryos, however, had a much larger amount,

Fig. 1.—Seventeen-day chick embryos from hens fed the low-B_{12} diet. Chick A was from egg injected with vitamin B_{12} prior to incubation and is normal in size and appearance. Chick B is from deficient egg.

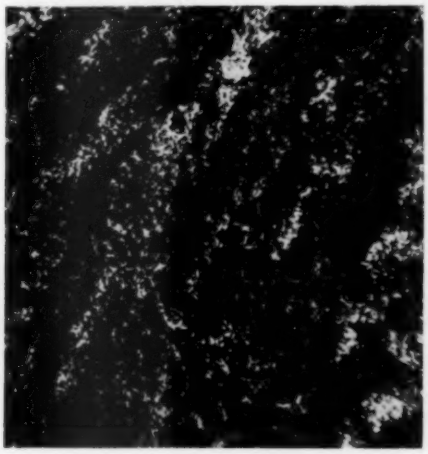
Fig. 3.—Striated muscle and subcutaneous tissue from 17-day B_{12}-deficient embryo. Muscle fibers are separated by the extreme edema. Hematoxylin and eosin stain; reduced about ⅕ from mag. × 180.

and the globules of fat were larger. In a few of the deficient embryos there were focal areas of degeneration in the skeletal muscles. The muscles in these deficient embryos appeared to be more cellular than those in the controls. This apparently may be explained by the fact that the muscles in the deficient embryos were smaller and had not developed to a degree equal to those of the controls.

The hearts of the deficient embryos were enlarged, and many showed gross hemorrhages (Table). The myocardium was pale in 62% of the cases. This color resulted from the presence of fat within the cardiac muscle fibers (Fig. 4). Essentially no fat was present in the myocardium of the control embryos. Focal areas of degeneration were present in the myocardium in some of the deficient embryos.

The liver in 81% of the embryos from hens fed the low-B_{12} diet was pale and frequently had focal areas of hemorrhage. In a normal embryo, or in one provided with vitamin B_{12}, at 17 days of age the liver was reddish-brown and microscopically resembled that in an adult chicken (Fig. 5). The hepatic cells contained a moderate amount of lipid. The vacuoles within the hepatic cells, as seen in the hematoxylin and eosin preparations, were small and more or less uniform. The fat vacuoles were more numerous in the vitamin B_{12}-deficient livers (Fig. 6). In focal areas the hepatic sinusoids were markedly dilated; in fact some appeared as small

Fig. 4.—Cardiac muscle from 17-day B_{12}-deficient embryo showing large amounts of fat. Sudan IV (green filter); reduced about ⅙ from mag. × 300.



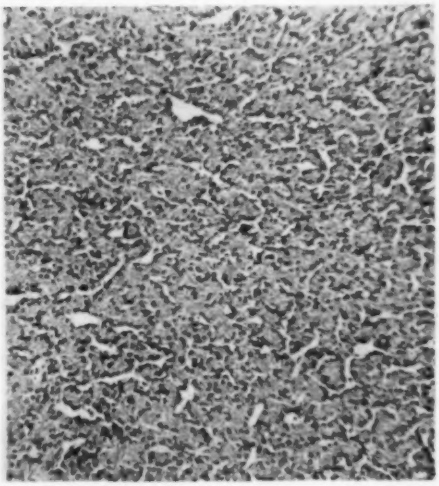


Fig. 5.—Liver from 17-day chick embryo. Egg was injected with vitamin B_{12} prior to incubation. Hepatic cells and sinusoids are normal. Hematoxylin and eosin stain; reduced about ⅙ from mag. × 180.

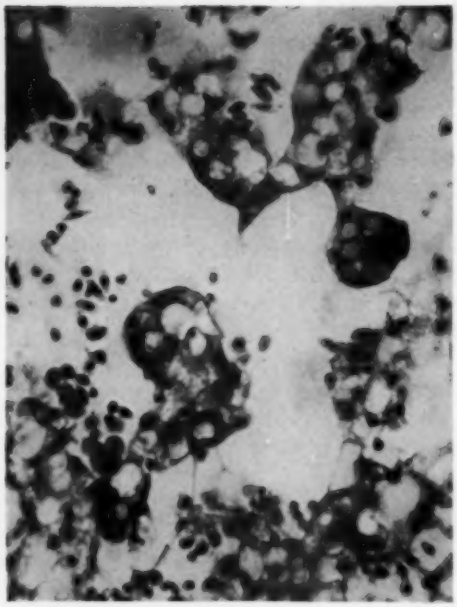
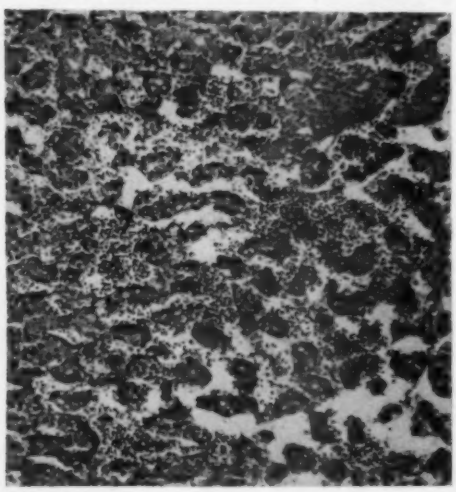


Fig. 6.—Liver from 17-day B_{12}-deficient embryo. Excessive and large fat vacuoles are present. The sinuses are very large; some appear cystic. Hematoxylin and eosin stain; reduced about ⅓ from mag. × 602.

cavities (Figs. 6 and 7). Red blood cells usually filled these spaces (Fig. 7). Large areas of necrosis were present in the liver (Fig. 8). These were located more frequently

Fig. 7.—Liver from 17-day B_{12}-deficient embryo. Dilated sinuses filled with blood. Hematoxylin and eosin stain; reduced about ⅓ from mag. × 120.



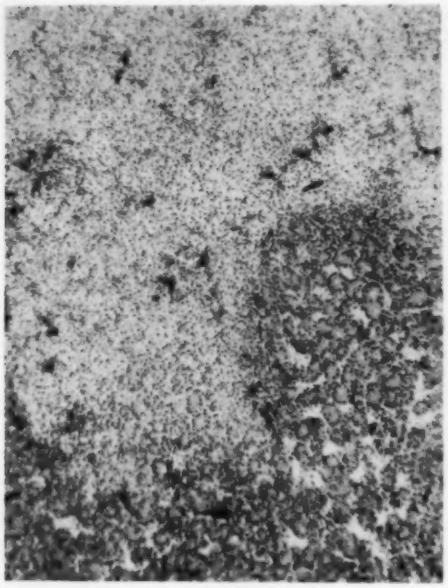
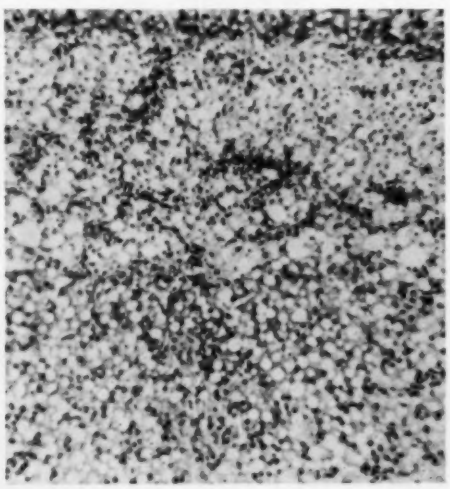


Fig. 8.—Liver from 17-day B_{12}-deficient embryo showing extensive necrosis. Note the absence of any inflammatory reaction. Hematoxylin and eosin stain; reduced about ⅓ from mag. × 110.

near the edge of the lobes. A zone of well-preserved hepatic cells was located between the necrotic area and the capsule of the liver

Fig. 9.—Liver from 17-day B_{12}-deficient embryo. The focal area of necrosis is separated from the capsule by a narrow zone of well-preserved hepatic cells. Many large fat vacuoles are present in the hepatic cells. Hematoxylin and eosin stain; reduced about ⅓ from mag. × 190.



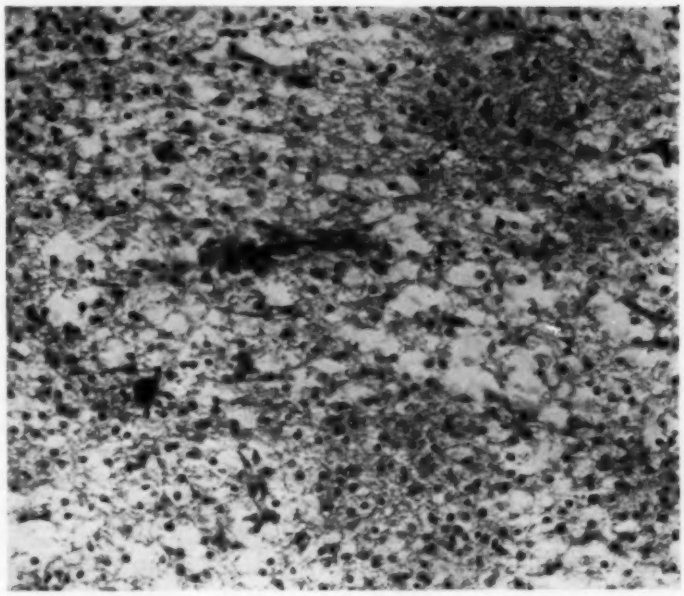


Fig. 10. — Area of degeneration in the brain of a 17-day B_{12}-deficient embryo. Hematoxylin and eosin stain; × 170.

(Fig. 9). These areas of necrosis varied in both size and shape; frequently they were observed macroscopically. There was no inflammatory reaction associated with this hepatic degeneration. Large numbers of red blood cells were present in some of the necrotic areas. No vascular occlusions were found.

In approximately half of the vitamin B_{12}-deficient embryos the kidneys were pale. Minute droplets of fat were present in the epithelial cells of the renal tubules in the normal kidney; however, the amount was greater and the droplets larger in the deficient kidney. Epithelial cells, leucocytes, and debris were presented in the lumina of

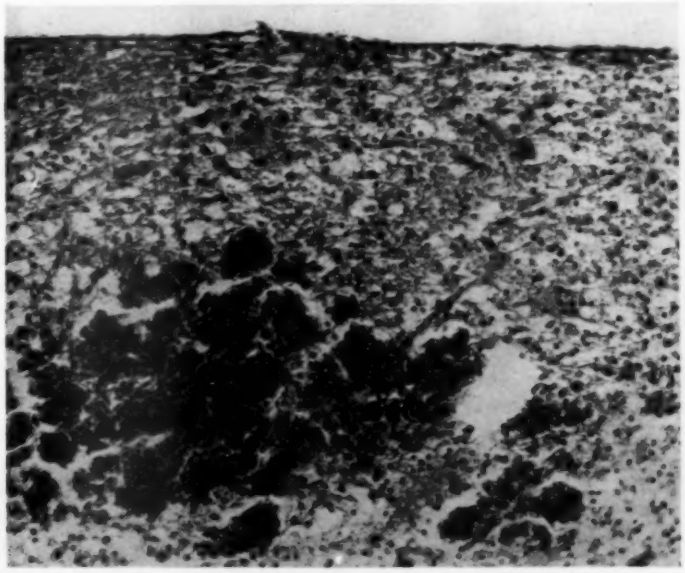


Fig. 11. — Area of degeneration in the brain of a 17-day B_{12}-deficient embryo with hemorrhage also present. Hematoxylin and eosin stain; × 120.

the renal tubules in the mesonephric portion of the kidney. Some of the tubules showed extensive degeneration of the cells lining the convoluted portion. Since these changes were present in both the normal and the deficient embryo, no significance could be attached to them. It may be recalled that the mesonephros is quite large by the 17th day, and degeneration normally has begun.

The wall of the intestinal tract in the deficient embryo was thinner than that of the normal, and hemorrhages were present. The esophagus, crop, proventriculus, gizzard, duodenum, ileum, cecum, and rectum were less mature in the deficient embryo than they were in both the normal and those receiving the vitamin B_{12} supplement. This immaturity was evident by the incomplete development of the epithelial glands and the smooth muscle. The intestinal tract of the 17-day-old deficient embryo was similar to that of a 14-day-old normal embryo. Sudan IV stains on the intestinal tract showed many fat droplets within the epithelial and smooth muscle cells. Control embryos had fewer fat droplets, and their size was smaller than those in the deficient embryos. The epithelium of the deep glands of the proventriculus had a larger amount of fat than that observed in any other portion of the gastrointestinal tract.

The epithelium lining the trachea and alveoli in the deficient embryo had more fat and the droplets were larger than those in the embryos given vitamin B_{12}. The stroma of the lungs was wider, and the pulmonary air spaces correspondingly smaller in the deficient than in the normal embryo. These variations are consistent with prematurity as previously discussed.

Twelve per cent of the deficient embryos showed gross hemorrhages in the brain (Table). The blood was in either the subarachnoid space or brain tissue. Four of the brains from the deficient embryos were studied histologically and showed local areas of degeneration (Fig. 10). The size of such areas varied widely. A similar area of degeneration was found in the spinal cord of one of the B_{12}-deficient embryos. Red blood cells were present in some of these areas of necrosis (Fig. 11). There were neither occluded vessels nor a cellular reaction about these foci of degeneration. No pathologic changes were observed in either the spinal ganglia or the peripheral nerves in these embryos.

The marrow in all the bones had relatively only a few cells. No variations were observed in the deficient and the normal embryos. No foci of extramedullary blood formations were observed in any of the tissues. No changes have been observed in the spleen.

The adrenal glands, gonads, and pancreas were premature, a feature so characteristic of other organs. No significant differences were observed in these glands in the normal and deficient embryos. The thyroid in a 17-day-old normal embryo had many follicles filled with colloid. The acini were lined by cuboidal epithelium. Few areas were present in which the cells were arranged in cord-like formation, a feature so characteristic of the earlier stages of development of this gland. In the 17-day-old deficient embryo there was a decrease in the number of follicles in the thyroid. The few that were present were usually very large. Many areas were present in which there was little, if any, evidence of follicle formation.

COMMENT

Embryos obtained from eggs produced by vitamin B_{12}-deficient hens have shown four pathologic processes that are not present in the embryos of either vitamin B_{12}-supplemented or normal embryos. These are (1) a decrease in size; (2) edema and hemorrhage; (3) focal areas of necrosis in the liver, brain, and spinal cord, and (4) a marked increase in fat in the parenchymatous tissues. The relation of these pathologic changes one to another is not known. Furthermore, the sequence of development of these lesions is unknown.

Seventy-four per cent of the 139 embryos examined in the present study showed gross evidence of pathologic changes, as shown in the Table. This is an increase over the number of deficient embryos reported pre-

viously by Ferguson and Couch [2] and may be explained by the fact that the hens were maintained on the low-B_{12} diet for a longer period of time than in the previous experiment. The rate of depletion of vitamin B_{12} in the hen varies.[2]

The pathologic changes occurring in the thyroid gland of the vitamin B_{12}-deficient embryos are most interesting. These will be reported in detail in a subsequent paper. A disturbance in the function of the thyroid gland may have been a factor in the retardation in growth of these deficient embryos. No histologic changes were observed in other glands of internal secretion. There are many factors that may either cause or contribute to the decrease in the rate of development of these embryos. The presence of fat in all the parenchymatous tissues in quantities greater than normal either would suggest some basic disturbance in lipid metabolism or may reflect only a secondary pathologic process, such as we frequently find following a variety of injurious agents, including metabolic disturbances and chemical agents. The relationship of vitamin B_{12} to lipid metabolism has been discussed by Ling and Chow.[4] These authors were unable to find any increase in fat in the liver of B_{12}-deficient rats. This observation is interesting in view of the tremendous amount of fat that we find in the livers of the B_{12}-deficient chick embryos. The presence of edema and hemorrhages in the chick embryo would suggest a possible disturbance in protein metabolism and in capillary permeability. The extensive pathologic changes in the liver of the deficient embryos would certainly result in the abnormal formation of the blood proteins.

The necroses found in the liver, brain, and spinal cord of the chick embryo are interesting in that no vascular occlusions were found that would account for their development. There is a similarity between the hepatic necrosis and the focal dilatation of the hepatic sinusoids in these chick embryos when compared with the hepatic lesions described by Furth and Sobel [5] in mice, which they attributed to hypervolemia. Wang and associates [6] did not find any histologic differences in the livers from B_{12}-deficient and B_{12}-supplemented rats. Stern and associates [7] noted a marked reduction of cytoplasmic basophilia in the liver from B_{12}-deficient weanling rats. Rasch and co-workers [8] concluded from a study of liver nucleoproteins in vitamin B_{12}-deficiency in the rat that "the clearest single effect found in animals placed on a B_{12}-deficient diet was the marked increase in total protein per liver cells." Biochemical studies † have shown both deoxyribonucleic acid and ribonucleic acid to be decreased per gram of tissue in deficient rats, although the total amounts per cell were little changed.

There may be no relation whatsoever between the therapeutic results obtained in clinical cases showing neurologic symptoms when treated with vitamin B_{12},‡ the hydrocephalus that occurs in the young vitamin B_{12}-deficient rats,[14] and the degeneration that is present in the brain and spinal cord of these vitamin B_{12}-deficient chick embryos. However, the fact that these phenomena do exist would suggest a possible relationship.

SUMMARY

The pathologic changes occurring in chick embryos obtained from vitamin B_{12}-depleted hens have been described. The lesions may be grouped into four categories: (1) decrease in size; (2) edema and hemorrhages; (3) focal areas of necrosis in the liver, brain, and spinal cord, and (4) a marked increase in fat in the parenchymatous tissues. The mechanism for the development of these lesions is unknown. The possible significance of these pathologic manifestations to the use of vitamin B_{12} in the treatment of neurologic diseases is discussed.

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Liver Damage in Children with Special Reference to Hepatic Cirrhosis

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Several workers, notably Radhakrishna Rao,[1] Gillman and Gillman,[2] and Trowell and Muwazi,[3] have drawn attention to the wide prevalence of hepatic cirrhosis among children of tropical countries and have laid emphasis on the part played by deficient diets in its pathogenesis. Although careful studies relating to hepatic cirrhosis in Ceylonese adults have been made,* little attention has been given to hepatic lesions which occur in infants and children in Ceylon.

An attempt has been made, in this study, to examine various types of liver injury and to evaluate the role of such injuries in the production of hepatic cirrhosis. The material consisted of 184 consecutive specimens of liver tissue (174 autopsy specimens and 10 liver biopsies) obtained from infants and children admitted to the Lady Ridgeway Hospital for children and the De Soysa Maternity Home, Colombo, whose ages ranged from 1 month to 14 years (average, 3.04 years). Histological examinations of these specimens showed some form of liver injury in 129 cases, an incidence of 70% (Table).

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From the Department of Pathology, University of Ceylon.

* References 4 and 5.

Types of Liver Injury Found in a Total of One Hundred Eighty-Four Specimens

	No.	Percentage
Fatty liver *	85	66
Early cirrhosis	23	18
Liver necrosis	5	4
Postnecrotic cirrhosis	9	7
Biliary cirrhosis	6	4
Syphilitic cirrhosis	1	1
Total	129	

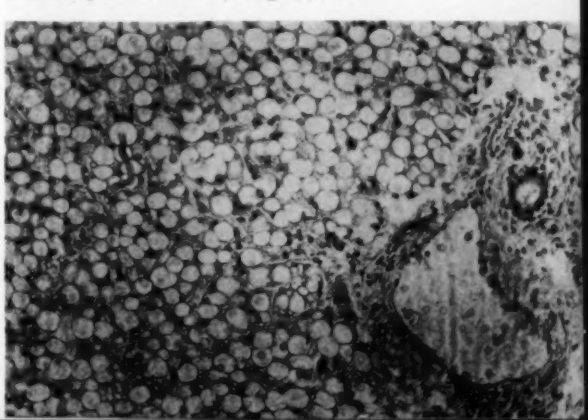
* Thirty-six of these showed early cirrhosis.

TYPES OF LIVER INJURY

(*a*) *Fatty Liver.*—This type appeared to be the most frequent type of hepatic injury, 66% of the specimens showing fat within liver cells. The ages of these children ranged from 3 months to 9 years, the average being 3.2 years. In 36 (42%) fatty infiltration was associated with the early stages of a cirrhotic process (see below), but in the remaining 49 (58%) fat within liver cells was unaccompanied by cirrhosis. In a large number of cases every liver cell was so distended with fat that the liver architecture was unrecognizable (Figs. 1 to 3), but in a few the fat was zonal in distribution.

A careful analysis of the case histories revealed that the fatty liver was associated

Fig. 1.—Extensive fatty infiltration. Liver architecture unrecognizable. Portal tract on right. Hematoxylin and eosin; mag. × 145.



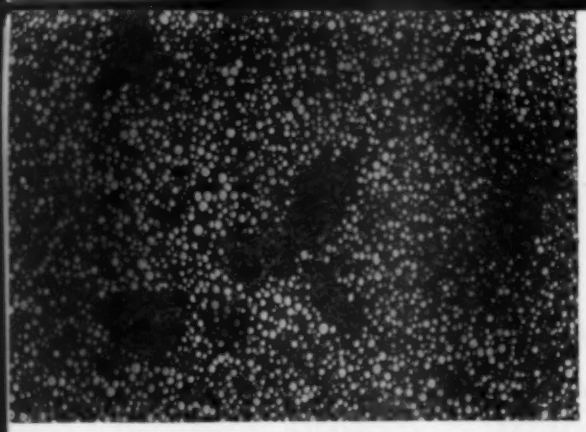


Fig. 2.—Heavy fatty infiltration. Cellular infiltration confined to portal tracts. Hematoxylin and eosin; mag. × 70.

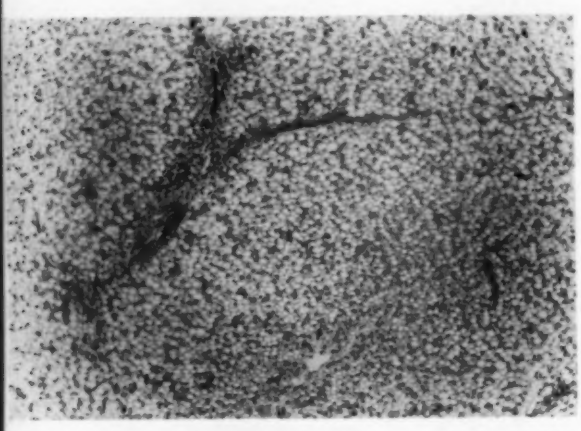
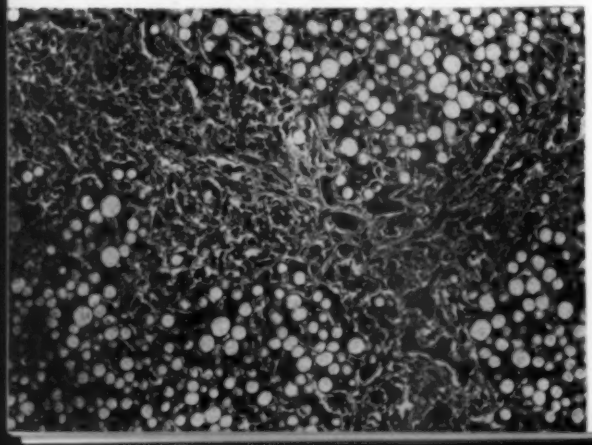


Fig. 3.—Heavy fatty infiltration with cellular bands traversing liver parenchyma. Hematoxylin and eosin; mag. × 70.

with a multiplicity of other pathological conditions such as bacterial infections, toxemias, malnutrition, and anemia. Pneumonia, lung abscess, dysentery, and tuberculosis were the most frequent bacterial infections, while Ascaris infestation and infantile diarrhea and vomiting were the most potent cause of toxemia. Almost all these children suffered from anemia and malnutrition.

Fig. 4.—Higher magnification, showing cellular character of the bands encircling lobules in a fatty liver. Hematoxylin and eosin; mag. × 145.



(*b*) *Cirrhosis.*—In 59 (58%) early cirrhotic lesions were seen. These were characterized by excessive cellular proliferation in the portal tracts or liver parenchyma, the cells consisting of lymphocytes, histiocytes, and fibroblasts supported on a delicate fibrillary stroma. Limitation of these cells to portal canals (Fig. 2) has been recognized by us as a very early stage of the cirrhotic process, and the spreading of these cells outward from the portal areas to the liver parenchyma in the form of bands of varying thickness (Fig. 3), as a later stage. Ultimately one or more liver lobules were encircled by such bands (Fig. 4). In this final stage the presence of proliferating bile ducts was a noteworthy feature. Although the portal canals and the liver parenchyma were very cellular, in none of these stages was there any recognizable mature collagen, and regeneration nodules were conspicuous by their absence.

The ages of these children ranged from 1 month to 14 years, the average age being 3.2 years. A noteworthy feature in this group was the absence of a previous history of jaundice. The associated pathological conditions such as bacterial infections, toxemias, malnutrition, and anemia were similar to those met with in the cases of fatty livers.

(*c*) *Liver Necrosis.*—Of the five cases in this group, two were massive necrosis ("acute yellow atrophy") (ages, 1⅔ years and 5 years, respectively); two focal (ages, 1 year and 3 months, respectively), and in a single case (age 2 years) the necrosis was confined to the centrilobular zones, as in infective hepatitis. Jaundice was present in both the massive and centrilobular types of necrosis but was absent in the two cases of focal necroses, one of which was associated with bacillary dysentery and the other (a marasmic infant of 3 months) with portal vein thrombosis.

(*d*) *Postnecrotic Cirrhosis.*—This type accounted for 7% of the liver injuries. This

condition was met with in slightly older children, whose ages ranged from 1½ years to 14 years (average, 5.6 years). All these cases either presented with or gave a previous history of jaundice and showed signs of liver failure, as well as varying degrees of portal obstruction. The histological picture was that of a multilobular cirrhosis characterized by the presence of mature collagen which traversed the liver parenchyma, separating off nodules of hyperplastic liver tissue (Fig. 5). Bile-duct proliferation was a conspicuous feature in this type.

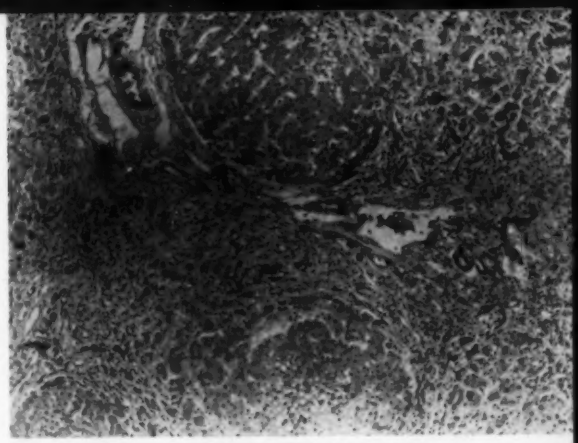


Fig. 5.—Postnecrotic cirrhosis with two hyperplastic liver nodules. Hematoxylin and eosin; mag. × 70.

(*e*) *Biliary Cirrhosis.*—In six cases (4%) with jaundice as the common feature, the histological appearance of the liver tissue conformed to the so-called biliary type of cirrhosis. Three cases were in young infants (a few days old, 5 months, and 9 months, respectively) who were afebrile. In the infant a few days old there was round-cell infiltration of the portal tracts, with slight distention of the biliary canaliculi. In the two remaining cases the hepatic lesion was one of advanced cirrhosis characterized by the presence of thick bands of collagen which traversed the liver parenchyma, separating off rather large lobules of liver tissue. There was marked proliferation as well as considerable distention of bile ducts with the formation of bile thrombi (Fig. 6).

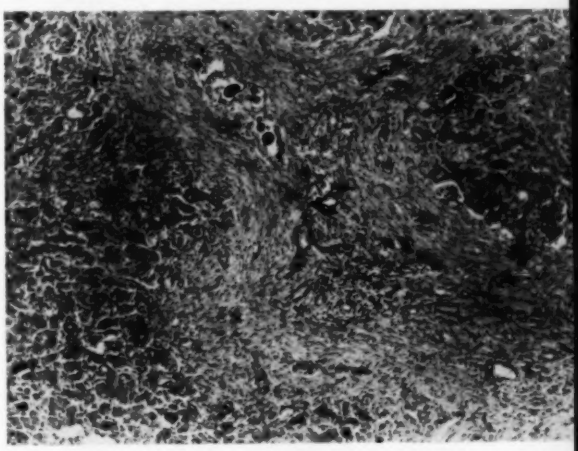
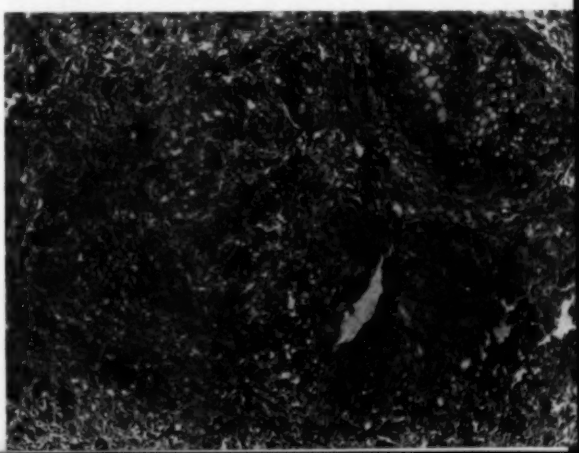


Fig. 6.—Late biliary cirrhosis. Biliary thrombi and mature fibrous tissue traversing liver parenchyma. Hematoxylin and eosin; mag. × 70.

The three other cases of biliary cirrhosis occurred in slightly older infants (1½, 1⅔, and 2 years, respectively), with long-continued pyrexia and in whom the livers were considerably enlarged. The histological features showed certain differences from the former types of biliary cirrhosis. The fibrocellular tissue, in addition to traversing the larger interlobular spaces, also extended into the liver parenchyma, separating off smaller groups of liver cells, which appeared to be isolated and surrounded by this tissue (Fig. 7).

Multinucleated liver cells laden with hemosiderin granules and hemopoietic foci in the liver parenchyma, features described by Dible and co-workers [6] as occurring in a group of cases termed by them "foetal and neonatal hepatitis," were not observed in the present series.

Fig. 7.—Cholangiolitic cirrhosis. Fibrocellular tissue separating off small groups of liver cells. Hematoxylin and eosin; mag. × 70.



COMMENT

The liver tissue in the present study was obtained from infants and children admitted

to the one and only children's hospital and to the largest maternity hospital in Ceylon. Most of the cases admitted to these two hospitals came from very poor homes, and there is no doubt, as revealed in this study, but that gross forms of liver damage occur with the greatest frequency among them, 70% in this series showing some form of liver injury. Of the various types of hepatic lesions listed in the Table, fatty liver is the most frequent, 66% showing, according to histological criteria, an excess of fat in liver cells. In 48 of these (56%) fatty infiltration was extensive, every liver cell being heavily laden with fat (Figs. 1 to 4). Such an extensive degree of fatty infiltration of the liver has also been previously reported in Ceylonese adults † and in a few Ceylonese children who presented with the syndrome of kwashiorkor.‡ Although nutritional disorders are frequently met with in Ceylonese children, the extreme form of malnutrition, kwashiorkor, is not so common as in tropical Africa. In a recent study [8] of the pattern of children's disease, as seen in this same hospital, it was found that 683 cases out of a total of 2168 admissions were nutritional disorders, but there were only 30 cases of kwashiorkor. That nutritional deficiency is not the only etiological factor in the causation of such an extreme degree of fatty change in the liver tissue studied by us becomes apparent from a study of the clinical history of these cases. Although these children showed varying degrees of malnutrition, they also presented other pathological states, such as anemia, bacterial infections, and toxemia, which are well-known to contribute to the production of a fatty liver. Helminthic infestation is so heavy that toxemia becomes a predominant feature in such cases. It would therefore appear that, in Ceylon, at any rate, several etiological factors operate in the causation of fatty liver. In laying too great an emphasis on malnutrition, tropical workers are apt to ignore infections and Ascaris infestation, which should also receive appropriate treatment, both prophylactic and curative, in order that the incidence of this type of hepatic injury may be reduced.

The term "cirrhosis" is applied to "an extensive diffuse scarring of the liver which has followed the destruction of much of the liver substance" (MacCallum [9]). There is general agreement that in hepatic cirrhosis parenchymal destruction and scarring accompanied by parenchymal regeneration are present, but extensive scarring and regeneration nodules constitute the terminal stages of a long evolutionary process. The early stages of this process have been described by Cameron and Karunaratne [10] in the experimental animal. In the production of cirrhosis by carbon tetrachloride they observed that the portal tracts at first became cellular. The cells, consisting chiefly of fibroblasts and histiocytes, gradually spread from the portal areas toward the liver parenchyma until liver lobules were encircled by this cellular tissue. Similar appearances have been depicted in human fatty livers in this study (Figs. 2 to 4) and have been regarded by us as those of early cirrhosis. We have also shown, in another group of children, the lesion of late or established cirrhosis characterized by mature connective tissue formation, liver regeneration, and bile duct proliferation (Figs. 5 to 7). These cases were accompanied by signs of portal obstruction and liver failure. These two types of cirrhotic lesions, the early and the advanced, appeared to be different in their etiology. The former were found chiefly in fatty livers without a history of jaundice; the latter occurred in cases with a history of jaundice, massive hepatic necrosis, and biliary obstruction.

The presence of early cirrhotic lesions in the fatty livers of infants and children raises the question whether such changes can result solely from the distention of liver cells with fat. Himsworth § has suggested that the hepatic cells distended with fat compress the lobular sinusoids, resulting in degenera-

† References 4 and 5.

‡ References 7 and 8.

§ Himsworth, H. P.,[11] pp. 68 and 69.

tion and disappearance of the central cells of the lobule. According to Himsworth, "The situation is analogous, though to a lesser degree, to that in repeated attacks of centrilobular necrosis; and in both cases a diffuse hepatic fibrosis results." This conclusion, which is based on the results of animal experiments, is hardly applicable to the present series, where other factors beside malnutrition, such as toxemias and infection, also operated in the causation of fatty liver. Liver-cell damage may have been caused by such toxins, with the consequent absorption of the autolytic products of dead cells resulting in the stimulation of cellular tissue in the portal canals to proliferate. The same average age incidence (3.2 years) for both fatty infiltration and early cirrhosis precludes the possibility that the early cirrhotic lesions that have been described were a late sequel to fat accumulation in the liver. If this were so, the average age of the children showing cellular infiltration of the portal tracts would have been higher than that of the cases with fatty infiltration.

Another important question which is worthy of consideration is whether these early cirrhotic changes are likely to progress to the terminal stages of established cirrhosis when mature collagen traverses the liver parenchyma, as in the case of experimental animals where it has been observed that prolonged fatty infiltration ultimately results in diffuse hepatic fibrosis.[12] According to Himsworth,‖ the fat content of the liver in rats needs to be as high as 30% to produce fibrosis within 100 days, while fibrosis is produced after 300 days when the fat content is only 15%. Although it was not possible to estimate the liver fat in our human cases, the histological appearances (Figs. 1 to 4) are indicative of a degree of fatty infiltration equal in severity to that of fatty livers produced by experimental methods. However, the present study does not lead us to presume that infants and children with fatty livers and incipient cirrhosis will ultimately suffer from the condition of established cirrhosis with attendant signs of portal obstruction. A considerable interval, which according to Dible[13] is 7 to 8 years, has to elapse before the young fibroblasts observed by us in the portal tracts and liver parenchyma (Figs. 3 and 4) form mature collagen. The average age for fatty liver disease and early cirrhosis being 3.2 years, the condition of established cirrhosis cannot be expected to occur earlier than the age of 10 years. If fatty liver disease, which is so frequently seen in the Ceylonese child, progresses, as in experimental animals, to well-developed cirrhosis, then hepatic cirrhosis should show a high incidence about the age of 10 years. Both clinical and autopsy records in the Lady Ridgeway Hospital show a remarkably low incidence of cirrhosis at this age period, such cases being only those preceded by a history of severe jaundice. Moreover, experimental work has shown that cirrhosis of the liver is a reversible condition (Cameron and Karunaratne[10]; Steinberg and Martin[14]). According to these workers, fibrous tissue laid down in the liver during the evolution of cirrhosis disappears on the discontinuance of the stimulus which induced these changes, and the liver returns to a normal state. There is undoubtedly a greater chance of a reversion to normality when the connective tissue is still cellular, as in the cases we have described. It is therefore reasonable to presume that a child with a fatty liver, as severe as in this series, either dies of the liver injury itself or succumbs to toxemia, which precedes or follows such hepatic injury. Adequate treatment results in the disappearance of both fat and cellular fibrous tissue. In either case, the sequel of established cirrhosis, which has been described as an inevitable event in experimental animals, does not follow in the case of these children. It would therefore appear that the statement so often made ¶ that the frequency of fatty infiltration in the malnourished children of the tropics accounts for the prevalence of infantile and juvenile cirrhosis in these regions is not applicable to infants and children of Ceylon.

‖ Himsworth, H. P.,[11] p. 66.

¶ Himsworth, H. P.,[11] p. 92.

Advanced cirrhotic changes in Ceylonese children, on the other hand, have a different etiology. Its incidence (15 cases, 11%) is lower than that of fatty liver disease and early cirrhosis (Table) and, unlike the two latter conditions, all cases of advanced cirrhotic changes gave a history of jaundice. These were met with during three different age periods: (1) in very young infants (under 9 months), (2) slightly older infants (between 1½ and 2 years), and (3) in children with an average age of 5.6 years. We believe that these groups are etiologically different.

The hepatic lesions in the first group were similar to those of biliary cirrhosis resulting from congenital atresia of the bile ducts. The early cellular infiltration of the portal tracts and the formation of mature fibrous tissue in the liver parenchyma (Fig. 6) appear to correspond to the early and late stages of the cirrhotic process described in human biliary cirrhosis by Gibson and Robertson [15] and by Moschcowitz.[16]

The two infants in the second group gave a history of a long pyrexial illness. Clinically and pathologically (Fig. 7) these cases resemble the type of cholangiolitic cirrhosis described by Watson and Hoffbauer,[17] who attribute the histological changes to a persistent leakage of bile from cholangioles as the result of altered permeability in these minute vessels brought about by infection. The cause of the infection, whether bacterial or viral, and the route of invasion, whether via the blood stream or biliary tract, are obscure.

The clinical features and the histological lesions in the third group (Fig. 5) leave very little doubt that these are cases following massive hepatic necrosis resulting from the action of the virus of infective hepatitis on liver tissue rendered vulnerable by the deficiency of protective substances.#

SUMMARY AND CONCLUSIONS

In a series of 184 consecutive histological examinations of liver tissue (174 autopsy specimens and 10 liver biopsies) of Ceylonese infants and children, whose ages ranged from 1 month to 14 years, with an average of 3.04 years, some form of liver damage was seen in 129 cases (70%).

Fatty liver was the most frequent type of hepatic injury (average age of onset, 3.2 years) and was noted in 85 cases (66%). In 48 of these (56%) the fatty change was extensive, all the liver cells being laden with fat, while in the remainder fat was zonal in distribution. As fatty liver was associated with other pathological states such as infections, toxemias, malnutrition, and anemias, it is not possible to single out any one factor such as malnutrition as its cause. Possibly several such etiological factors operate in the production of fatty liver in childhood.

There appears to be a tendency for early cirrhotic changes to develop in fatty livers, but there is no evidence that these changes are a direct sequel to distention of liver cells with fat. Such changes only represent the early stage of evolution of hepatic cirrhosis and are probably reversible, and there are no reasons to believe that they progress to the condition of established cirrhosis.

Established cirrhosis, on the other hand, follows jaundice resulting from massive hepatic necrosis or biliary obstruction. Massive necrosis is possibly a complication of infective hepatitis occurring in liver tissue which is rendered vulnerable by the deficiency of protective amino acids. Biliary obstruction is caused either by congenital obstruction of the bile ducts or by infection of the cholangioles. The cause of infection, whether bacterial or viral, and the route of invasion, whether via the blood stream or biliary tract are obscure.

Syphilitic cirrhosis accounted for only 1% of liver injury.

Prof. G. R. Cameron, F.R.S., of University College Hospital, London, gave valuable suggestions; Prof. C. C. de Silva, Professor of Pediatrics, University of Ceylon, and Dr. Stanley de Silva, Visiting Physician, Lady Ridgeway Hospital, provided the material for examination; Mr. K. M. M. Michael supplied the photomicrographs, and Messrs, J. C. Fernando and P. D. S. Amerasekera gave technical assistance.

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Studies in Rheumatic Fever

I. The Clinical Significance of the Aschoff Body Based on Morphologic Observations

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In a clinical sense, the term "chronic inflammation" is applied to disease processes characterized by a relatively long duration with or without acute exacerbations. Within the spectrum of the chronic inflammatory processes the granulomas form a special group. Tuberculosis, syphilis, leprosy, mycoses, and sarcoidosis are examples of this group. The tissue response is often pathognomonic, enabling the exact nature of the condition to be recognized. Such processes may be designated as "chronic specific" on the basis of the characteristic tissue alterations. If the etiology can be established, the specificity of the pathological changes is further strengthened.

The tissue response in these conditions undergoes a life cycle which tends to correlate with the clinical course. This is typified by the evolution of the tubercle. Thus, morphologic patterns assumed to represent active disease in the host are usually accompanied by clinical manifestations. With histologic evidence of diminution in the intensity of the process, clinical activity may correspondingly subside. Clinical quiescence in most instances denotes tissue healing.

Rheumatic heart disease is also of long duration and is characterized by a tissue response predominantly productive in character. The etiology of the disease is not conclusively established, but since the Aschoff body is generally regarded as the pathognomonic lesion of the process, rheumatic disease may be accepted as a member of the "chronic specific" group of granulomatous inflammation. Whether or not the Aschoff body offers a basis for correlation with the course of the disease it represents has not been conclusively established as yet.

HISTORICAL REVIEW

Aschoff [1] was the first to indicate the ultimate transformation of the rheumatic lesion into connective tissue, thus defining a temporal sequence of events. Geipel [2] studied five fatal cases of rheumatic carditis and showed that the Aschoff body became visible between the fifth and the sixth week after the onset of the disease. Its appearance was preceded by an exudative phase with infiltration of polymorphonuclear leucocytes. The similarity of the acute lesions to abscesses was noted by Bracht and Wächter.[3] These authors described the Aschoff body as being composed primarily of polymorphonuclear leucocytes and lymphocytes followed by larger cells in later stages of development. Similar observations were made by Fraenkel [4] and MacCallum [5] and led Von Glahn [6] to state that the masses of leucocytes, lymphocytes, and plasma cells present were as prominent and distinctive as the Aschoff body. Talalaeff [7] recognized an early acute exudation which extended into the second or third week, followed by a proliferative phase of

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from one to six months terminating in sclerosis. It becomes apparent that the Aschoff body may represent any stage "from the active acute exudative phase, which is less common, through the exudative lymphocytic stage to the almost pure proliferative condition in which large mononuclear and multinucleated cells are chiefly found" (Clawson [8]).

The role of the connective tissue in the development of the Aschoff body was noted very early by students of rheumatic disease. Mallory,[9] Huzella,[10] Aschoff and Fraenkel,* and others noted the presence of altered collagen fibers. Klinge [11] advanced the concept of changes in the interfibrillar ground substance of the connective tissue as the earliest change preceding the development of the mature lesion. He observed that at the end of the second week of illness the ground substance becomes intensely eosinophilic, resembling fibrin tinctorially, and called it fibrinoid. As the classic lesion develops, the fibrinoid and all cellular elements are replaced by connective tissue. Gross and Ehrlich [12] confirmed the collagen fiber damage as the earliest tissue manifestation of rheumatic disease but could not demonstrate fibrinoid with any degree of constancy. These workers [13] attempted to correlate the developmental sequence of tissue changes with the clinical course. They identified seven main histological patterns, all minor variants of the life cycle of the rheumatic lesion. Fresh verrucous lesions, pericarditis, and "acute inflammatory phenomena in the myocardium, valve rings, and leaflets" were anatomical signs of activity. However, the authors emphasized that some stages in the evolution of the Aschoff body may be absent or abbreviated, or may appear in reverse order, depending on the tissue reactivity. In addition, they stressed the presence of inflammatory cellular infiltration of the myocardium and the unpredictable demonstration of the Aschoff body. Thus, only 39% of 161 cases showed pathognomonic changes. Depending upon the paper cited, the percentage of Aschoff bodies noted in postmortem material of a given series of rheumatic hearts ranges from 32 to 94. This striking discrepancy is largely due to the nature of the sampling and morphological criteria used to establish the diagnosis of rheumatic fever.

Recently procedures have been developed for the correction of rheumatic mitral valvular disease. These techniques require the removal of part or all of the left auricular appendage, making this tissue available for study. Numerous reports have demonstrated that Aschoff bodies or Aschoff cells may be noted in these specimens. Since such findings have been largely construed as evidence of rheumatic activity, this connotation has been applied by various investigators. The accompanying Table [14] indicates the broad percentage range of frequency of occurrence. In all instances the preoperative diagnosis had been "rheumatic heart disease, inactive." Thomas and others [15] have compared the lesions in the appendages with those in the rest of the heart in patients dying of rheumatic heart disease. They established a high degree of correlation and concluded that the appendageal findings reflect the status of the heart as a whole. Aschoff bodies in the appendage are usually endocardial or subendocardial in location. Denst and co-workers [16] warn against interpreting the thickened

Incidence of Aschoff Nodules in Surgically Removed Auricular Appendages

Author, Date	No. of Cases	Incidence of Aschoff Nodules	
		No.	Per Cent
Pinninger (1951)	15	10	67
Waaler (1952)	28	8	28
Kuschner and others (1952)	11	4	36
Sabiston and Follis (1952)	43	32	74
Björck and others (1952)	18	8	44
Janton and others (1952)	88	14	16
Kuschner and others (1953)	28	14	50
Enticknap (1953)	71	29	41
Decker and others (1953)	183	83	45
Thomas and others (1953)	40	22	55
McKeown (1953)	53	24	45
Denst and others (1954)	75	21	28
Luse and others (1954)	77	32	41.6
Elster and others (1954)	15	9	60
Manchester and others (1955)	35	13	37.1
Present series	400	75	18.8
Total	1,180	398	33

* Aschoff, L., and Fraenkel, E., in discussion on Huzella.[10]

endocardium of the appendages as indicative of healed rheumatic disease, since the endocardium in this location, under normal conditions, is thicker than in other areas of the heart. This might also explain why Aschoff bodies are more frequently found in this location in the endocardium than in other areas of the heart.

The correlation of the tissue findings with the clinical stage of the patient postoperatively has been unrewarding. Attempts have been made [17] to relate the histological data with variations in sedimentation rate, cardiac rhythm, fever, ECG, antistreptolysin, and C-reactive protein titers, with doubtful significance. Histologically "active" patients failed to show any increase in operative morbidity or mortality, which would have been expected had the process been "active" clinically. An intensive review of the current literature clearly emphasizes the sharp discrepancies between the morphological changes and the clinical state of the patient. Unless the natural history of rheumatic disease has changed or the signs, symptoms, and laboratory procedures are inadequate, a reevaluation of the validity of the morphological criteria used as a measure of the clinical course must be considered. The purpose of this paper is to present a review of 400 consecutive left auricular appendages removed surgically as they relate to the problems discussed.

MATERIAL AND METHOD

A total of 400 biopsy specimens from left auricular appendages removed during the course of mitral valve surgery were studied. In all cases the preoperative diagnosis was "chronic mitral valvular disease of rheumatic origin." Clinical evaluation included detailed electrocardiographic and roentgenological procedures. Most patients were subjected to cardiac catheterization for pressure studies, estimation of blood volume, and oxygen saturation. Preoperative blood samples were analyzed for complete blood cell count, hematocrit value, sedimentation rate, urea nitrogen, C-reactive protein, antistreptolysin O titer, and protein patterns (electrophoresis [18]). All cases were considered clinically inactive prior to surgery. The serological and sedimentation rate studies were repeated during the postoperative period. Careful roentgenological measurements of heart size were also carried out postoperatively.

The biopsy material was immediately placed in ice-cold isotonic saline for transportation to the laboratory. The tissue was divided in equal parts. One part was fixed in modified Carnoy solution for four hours and then placed in 80% alcohol for routine processing. The other part was used for frozen sectioning or fixation in neutral formalin (4%). After paraffin embedding, serial sections were made at 3μ to 5μ in thickness. The ultrathin section adapter for the Spencer microtome allowed for some sections at 1μ to 3μ. Routine stains were hematoxylin and eosin, elastica-Van Gieson, Holmes' silver technique, toluidine blue, and periodic acid-Schiff procedure. Histochemical methods were applied to study the alterations of the ground substance. Phosphatases, esterases, lipases, and dehydrogenases were also evaluated. These detailed histochemical studies will be analyzed in a separate report.[19]

OBSERVATIONS

Since the Aschoff body or nodule, when properly identified, is generally regarded as the most characteristic finding of rheumatic disease, it was selected as the stigma of the process. On the basis of this fact, our material was divided at first into two main categories: appendages with Aschoff bodies and appendages without Aschoff bodies. As to the former, it has been pointed out that the Aschoff body is not a static structure. The proliferative phase is preceded by an exudative phase; "nuclear ghosts" can be found in senescent nodules, and recent nodules show changes of the collagen fibers and ground substance which are absent in older lesions. This variability in cellular and collagen structure was carefully scrutinized in our material. Accordingly, appendages showing presence of Aschoff bodies were subdivided into two distinct groups: those revealing alterations consistent with the early lesion and those displaying senescent changes. Degeneration of myofibrils and focal or generalized interstitial exudative changes were additional prominent and distinctive features of the former group, and appendages with these characteristics were thought to represent active rheumatic disease. In contrast, the appendages displaying senescent nodules and other manifestations of chronic carditis were considered as indicating a healed or healing rheumatic process.

More exactly, the rheumatic process was thought to be active when an exudative type of inflammatory reaction was detectable in the nodule, in the endocardium, or in the myocardium (independently from mural thrombosis); when the structure of the characteristic cells of the nodule was well made out; when the collagen fibers and ground substance were altered, and when the myofibers showed degenerative change. Since these unequivocal signs of acute process were in every instance accompanied by alterations (fibrosis, scarring, collagen hyalinization) indicating a chronic lesion, this combination of recent and old changes was interpreted as representing an exacerbation of chronic rheumatic heart disease. In the absence of these changes and in the presence of senescent Aschoff bodies and of myocardial or endocardial fibrosis, the rheumatic process was thought to be in a healed or healing phase. Since in all instances the clinical diagnosis was chronic rheumatic heart disease and this diagnosis was substantiated by the findings at surgery, biopsy specimens showing endocardial or myocardial fibrosis were placed in this category even in the absence of Aschoff bodies.

The tissue response in each of the two categories—active rheumatic carditis and healed or healing rheumatic carditis—was correlated with the preoperative and postoperative condition of the patient.

A. Active Rheumatic Carditis

Of the 400 auricular appendages examined, only 8 (2%) fell in the category of active rheumatic carditis. The morphology of the Aschoff body in all these cases indicated that the process was recent (Figs. 1 to 6). The lesion was located in the endocardium or, more commonly, in the subendocardial layer of loose connective tissue (Figs. 5 and 6). Additional nodules were found in the myocardium (Fig. 4) in two cases and adjacent to the wall of small intramyocardial branches of the coronary arteries in one case. Besides the disorganization of the fibrous tissue within the nodule, with swelling, eosinophilia, and granular degeneration of collagen fibers, which was noticeable in all cases, in three instances fibrinoid alteration of the ground substance was detected in endocardial and myocardial nodules. In another instance the same type of alteration was present in the myocardial stroma, and in another one it was

Fig. 1.—Early perivascular, intramyocardial Aschoff body showing extensive fibrinoid alteration of the ground substance and incipient inflammatory response, including giant-cell formation. There is concurrent hypertrophy of myofibrils with nuclear giantism and focal intervening fibrosis (auricular appendage SE-156, interpreted as reactivated rheumatic carditis). Hematoxylin and eosin; reduced about ⅝ from mag. × 175.

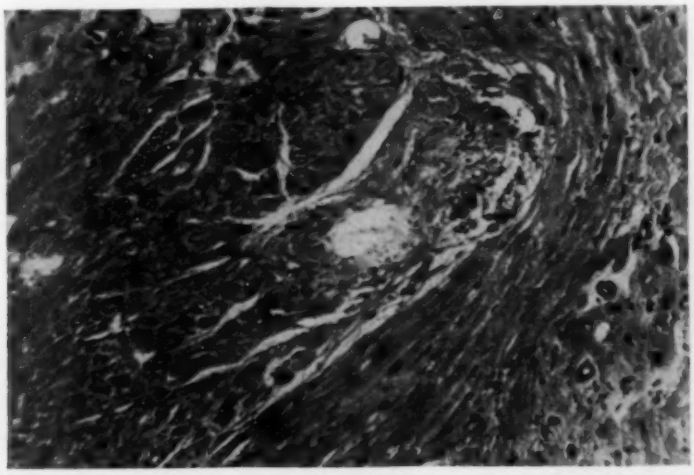
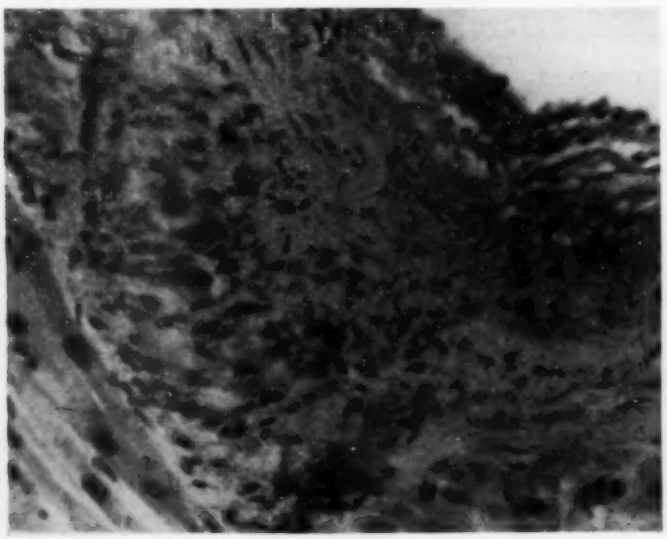


Fig. 2.—Diffuse exudative inflammatory reaction (in the absence of thrombosis) extending into early Aschoff bodies. Collagenized mural endocardium (auricular appendage SE-148, interpreted as reactivated rheumatic carditis). Hematoxylin and eosin; reduced about ⅔ from mag. × 175.

present in the wall of small blood vessels and had extended to involve the surrounding periadventitial stroma (Fig. 1). Metachromasia of the ground substance in or about the Aschoff body, either focal or diffuse, could be found in the absence of fibrinoid. An exudative inflammatory reaction with polymorphonuclear leucocytes, mostly of the eosinophilic type, within the Aschoff body (Fig. 3), in the endocardium (Fig. 2), or in the myocardium was an added feature of these eight cases. In four cases there was associated damage of myofibers, ranging from vacuolation and loss of striations to massive necrosis. One of these cases showed concurrent mural thrombosis with early organization and infiltration by lymphocytic cells involving the endocardium and the inner

Fig. 3.—Exudative inflammatory reaction, mostly by eosinophilic granulocytes within and around an early subendocardial Aschoff body (auricular appendage SE-183, interpreted as reactivated rheumatic carditis). Hematoxylin and eosin; reduced about ⅓ from mag. × 360.



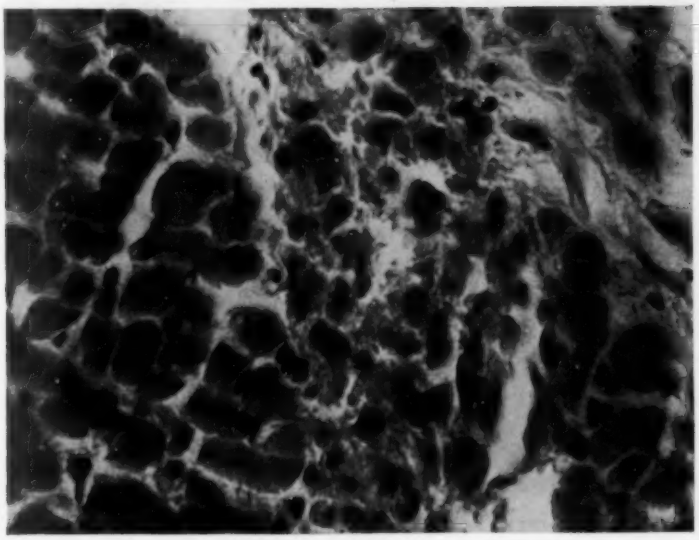


Fig. 4.—Collagen fiber degeneration in early intramyocardial Aschoff body (auricular appendage SE-131, interpreted as reactivated rheumatic carditis). Hematoxylin and eosin; reduced about ⅓ from mag. × 360.

Fig. 5.—Early subendocardial Aschoff body showing collagen alteration and incipient giant-cell formation (auricular appendage SE-146, interpreted as reactivated rheumatic carditis). Hematoxylin and eosin; reduced about ⅔ from mag. × 360.

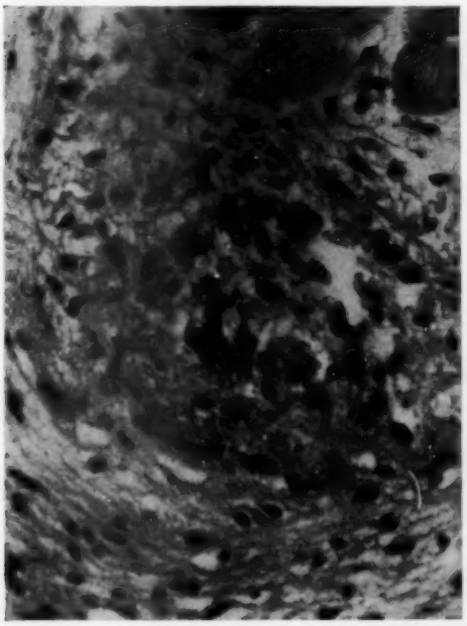
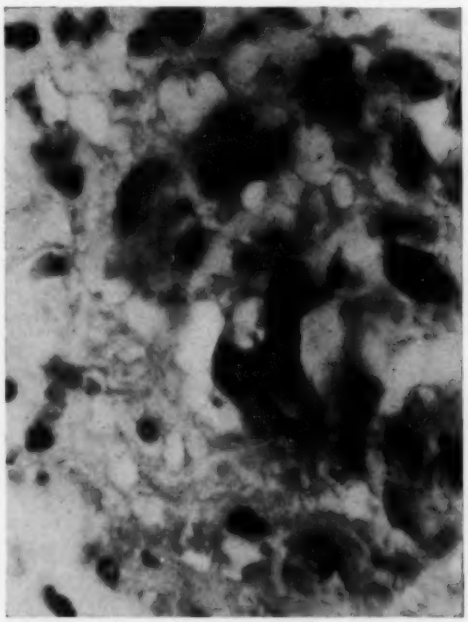


Fig. 6.—The same Aschoff body at higher magnification displaying characteristic "owl-eyed" structures. Reduced about ⅔ from mag. ×740.



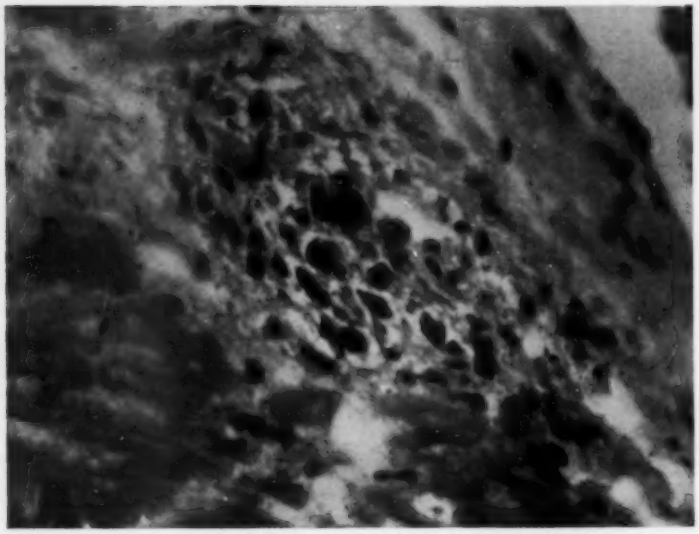
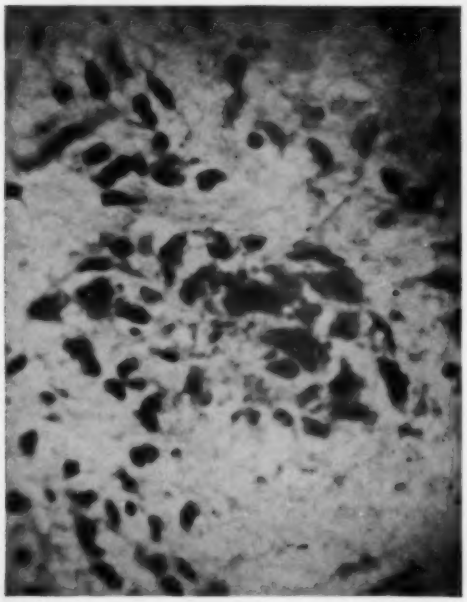


Fig. 7.—Senescent subendocardial Aschoff body displaying loss of boundaries between nucleus and cytoplasm in the individual cells and absence of nucleoli (auricular appendage SE-161, interpreted as healing rheumatic carditis). Hematoxylin and eosin; reduced about ⅓ from mag. ×360.

Fig. 8.—Senescent Aschoff body showing rarefaction of cells, replacement by connective tissue, karyorrhexis, karyolysis, and loss of cellular details: Ghost cells of Gross and Ehrlich (auricular appendage SE-163, interpreted as healing rheumatic carditis). Hematoxylin and eosin; reduced slightly from mag. × 360.



portion of the subjacent myocardium. Although in these eight cases the most striking manifestations were those of an acute process, there coexisted senescent Aschoff bodies of the "fibrillar" type (Gross and Ehrlich), endocardial thickening and fibrosis, increased interstitial connective tissue, focal myocardial scarring, and blood vessel walls (in three cases) which were also thickened and sclerotic. This association of chronic and acute lesions was thought to be consistent with a condition of old rheumatic carditis with recent reactivation.

B. Healed or Healing Rheumatic Carditis

Myocardial or endocardial fibrosis were constant features of the 392 cases of this group, 67 of which (16.8%) showed Aschoff bodies of the senescent type (Figs. 7 to 9). In every instance the Aschoff bodies were embedded in the endocardium or in the subendocardial layer of loose connective tissue. In four cases there coexisted nodules in the myocardium, and in four others nodules were found adjacent to the wall of blood vessels. In some instances, only 1 or 2 nodules could be found; in some others, as many

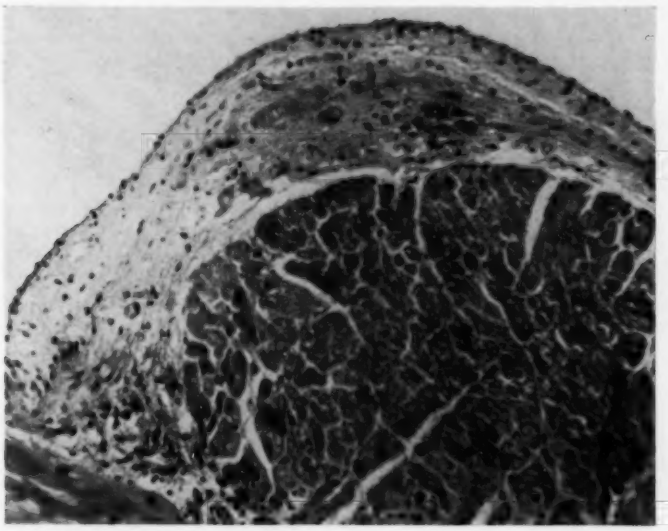


Fig. 9.—Senescent Aschoff bodies in collagenized endocardium showing (lower left) invasion by streams of fibroblastic cells (auricular appendage SE-194, interpreted as healing rheumatic carditis). Hematoxylin and eosin; reduced about ⅓ from mag. × 80.

as 8 or 10, but no attempt was made in this series to grade the lesion according to the frequency with which Aschoff bodies were encountered or to characterize the lesion according to the various forms of Gross and Ehrlich's classification. Our attention was focused instead on the characteristics of the cells within the nodule and the behavior of the ground substance within and around the nodule. In none of the cases of this group was evidence found of fibrinoid alteration of the ground substance in the nodule, in the wall of the blood vessels, or in the connective tissue of the endocardium or myocardium. In the absence of fibrinoid alteration, persistence of metachromatic properties of the ground substance was frequently noticed. Within the nodule the collagen fibers were swollen, fragmented, or fused together in an amorphous mass of dull eosinophilic material, in which there were embedded lymphocytoid cells and large mononuclear or multinuclear cells of the Aschoff type. The latter rarely showed the characteristic "owl-eyed" nuclei. The nucleus was oftener pyknotic than vesicular and resembled, in many respects, the "ghost nuclei" described by Gross and Ehrlich and interpreted as indicating senescent change. The myofibers were hypertrophic and showed nuclear giantism; less frequently and in limited areas they were atrophic or vacuolized. Regressive changes in myofibers were found in 50 cases. In 12 of these cases there were also rather severe vascular changes, and it is possible that defective blood supply may have contributed to the muscular damage. The small- and middle-sized intramyocardial branches of the coronary arteries were most frequently affected, with intimal thickening and sclerosis and narrowing of lumina. At times the entire vessel wall was thickened and sclerotic and the process extended into the periadventital fibrous connective tissue, which showed swelling and fragmentation of fibers and bright eosinophilic stain. In all cases the stroma in between the myofibers was increased in amount (Fig. 10) and the endocardium was markedly thickened. The thickening of the endocardium was either focal or diffuse and was due to proliferation of both collagen fibers and elastic fibers (Figs. 11 and 12). The collagen fibers for long stretches showed a bright eosinophilic stain, with loss of the natural wavy appearance, interpreted as evidence of regressive change, which often extended into the subendocardial layer of loose

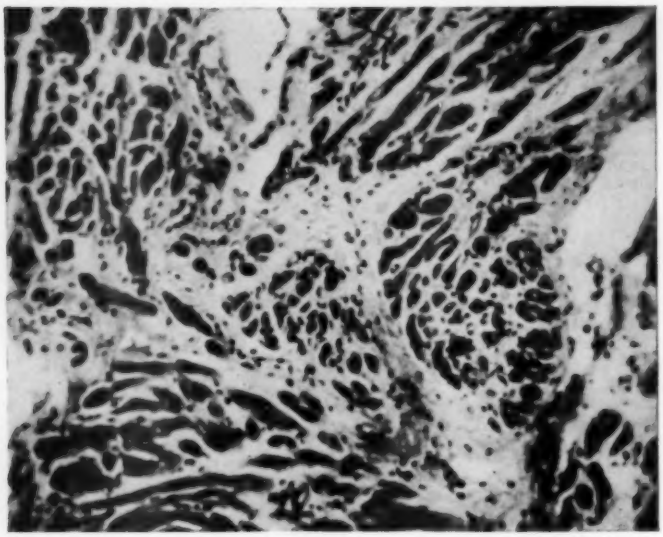
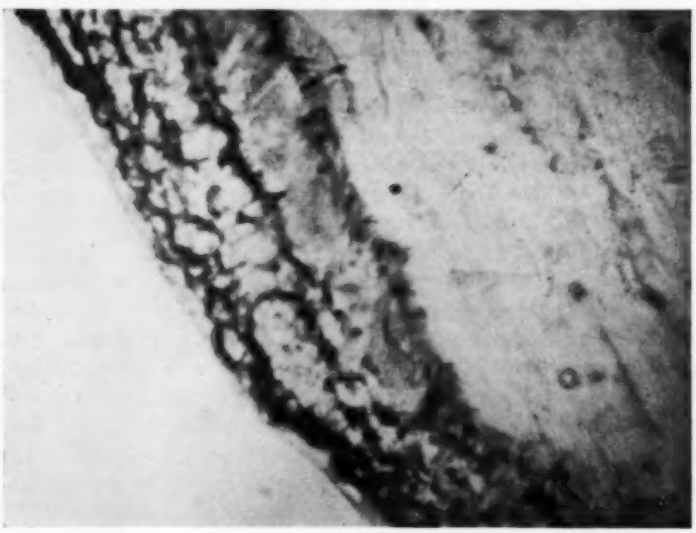


Fig. 10.—Myocardial fibrosis and scarring in the absence of Aschoff bodies (auricular appendage SE-173). Hematoxylin and eosin; reduced about ⅓ from mag. × 80.

connective tissue. The elastic fibers were also swollen, fragmented, or granular in appearance. In 40 cases the endocardial elastosis and collagenization were accompanied by increased cellularity (fibroblasts, histiocytes). In the remaining 352 cases mesenchymal cells were scarce or apparently absent.

Large basophilic cells with ragged edges and darkly stained nuclei, either sparse or irregularly aligned, were noticed in a few cases in the thickened endocardium. These cells resembled to some extent the characteristic cells of the Aschoff nodule. Although we realize that perhaps they corresponded

Fig. 11.—Mural endocardium of normal auricular appendage showing a network of elastic fibers and fibrous connective tissue. Elastic-Van Gieson stain; reduced about ⅓ from mag. × 175.



to the cellular structures that others had included in the consideration of "possible" or "probable" Aschoff nodules, since the lesion was equivocal and its significance was not clear, specimens with this type of alteration were considered as negative for Aschoff bodies. This might explain the lower incidence of Aschoff bodies in our series as compared with the higher figures reported by other investigators in the evaluation of comparable material. Endocardial and, less often, myocardial infiltration by lymphocytes, either

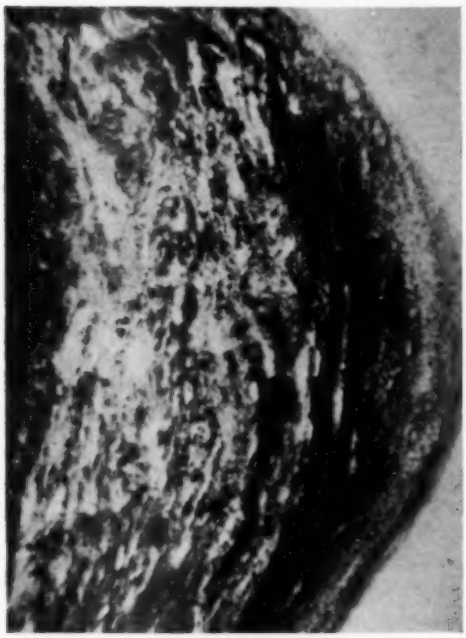


Fig. 12.—Mural endocardium of auricular appendage in healing rheumatic carditis displaying marked fibroelastosis and regressive changes both of collagen and elastic fibers (auricular appendage SE-136). Elastic-Van Gieson stain; reduced about 2/5 from mag. × 175.

sparse or in clusters, was found in 65 cases, and in every instance there was concurrent mural thrombosis. These inflammatory cells were regarded not as evidence of rheumatic activity, but rather as a nonspecific reaction elicited by the thrombi. In a few instances the thrombi were recent and consisted of laminated masses of fibrin containing erythrocytes and leucocytes. In the majority of cases they were old and showed advanced organization and abundant hemosiderin deposits.

In relation to the debated point of the origin of the multinucleated cell of the Aschoff body from nonmyogenic mesenchymal cells or from myofibrils, of significant interest is the finding in the present series of the co-existence in the same specimen of two distinct and well-characterized types of giant cells, one type suggesting myogenic origin and the other a nonmyogenic origin. The morphology of these cells and their significance will be analyzed in a separate report.[20]

C. Clinical-Pathological Correlation

A detailed review of the clinical findings in these 400 cases is beyond the scope of this paper. It is sufficient to state here that on the basis of the changes noted in the eight cases of active carditis one would have expected some clinical evidence of activity. In the absence of such manifestations, consideration must be given to the possibility that the widespread use of therapeutic agents prior to cardiac surgery may have modified the host response without affecting the underlying process. This assumption finds support in the recent demonstration of persistence of streptococci, in the absence of overt disease, in animals receiving cortisone.[21] The laboratory studies and x-ray measurements did not correlate with the histological picture. Of all the tests, only the sedimentation rate appeared to relate to the tissue changes. The postoperative course failed to reveal, in all cases studied, the evidence of recrudescence of the rheumatic process, including the eight cases considered to be histologically active.

COMMENT

Our observations indicate that the Aschoff body per se does not express activity of the rheumatic process. Consideration of its life cycle is imperative in any attempt to correlate clinical and pathological manifestations. Discrepancies in the identification of the Aschoff body and in its significance can be expected to arise until it is generally understood that this entity undergoes a life cycle. In the acute phase the earliest injury

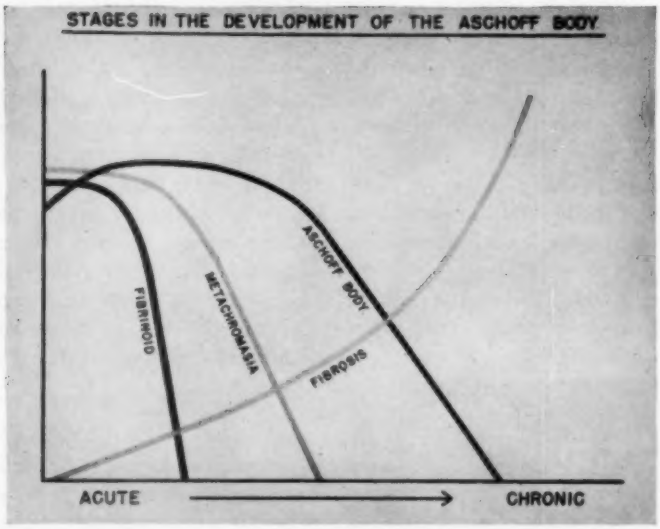


Fig. 13.—Graphic representation concerning the development of the Aschoff body as determined by histochemical methods.

is a fibrinoid alteration of the ground substance within the Aschoff body and the connective tissue of the heart. This fibrinoid material consists mainly of a richly metachromatic acid mucopolysaccharide and a protein component. Exudative inflammatory reaction, myofiber necrosis, swelling, and fragmentation of the collagen fibers are associated features of the early phases of the process. The transition to the chronic phase is characterized by a progressive disappearance of the above alterations, the appearance of senescent changes within the Aschoff body, progressive endocardial and myocardial fibrosis, and final scarring. As the process ages, metachromasia of the ground substance may persist, owing to the accumulated acid mucopolysaccharide, in the absence of fibrinoid. This chain of events is represented in Figure 13 in diagrammatic form. Our observations indicate further that reactivation of the process is accompanied by the appearance of fresh lesions, as shown by the association in our active carditis group of old and recent alterations.

Twenty-two patients included in these series died shortly after surgery and were submitted to postmortem examination. According to the findings in the appendage, 21 of the cases had been classified as healed or healing rheumatic carditis and 1, as active rheumatic carditis. Except for one case in the healed or healing rheumatic carditis group in which multiple sections from different segments of the heart failed to reveal presence of Aschoff bodies, which had been detected in the appendage, in all remaining cases the findings in the appendage compared exactly with those of other portions of the heart; this is in agreement with the observations of others (Thomas and co-workers [16]; Decker and co-workers [22]) and adds evidence in support of the concept that the auricular appendage is representative of the heart as a whole.

Previous investigators have attempted to correlate the occurrence of mural thrombosis with the stage of the rheumatic process. De la Chapelle, Graef, and Rottino [23] concluded that thrombosis develops more frequently in active carditis; on the contrary, Weiss and Davis,[24] Söderström,† Biörck and co-workers,[26] and Decker and associates[22] found that the incidence of thrombosis increases with the subsiding of the rheumatic process. In agreement with the observations of McGoon and Henly [27] no relation could

† Söderström,[25] cited by Decker.[22]

be seen in our series between the occurrence of thrombosis in the appendage and the stage of the rheumatic process, as determined by microscopic evaluation. As clearly expressed by the graph (Fig. 14), an arrangement of patients according to age group indicates in our series also a progressive decline of microscopic activity with advancing age.

In the present series of auricular appendages studied, the incidence of unequivocal Aschoff bodies is less than the average frequency of all series previously reported. This is not too surprising, since the disease proc-

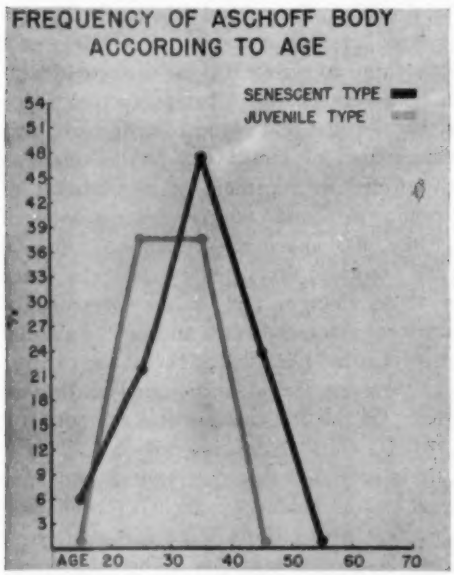


Fig. 14.—Frequencies of occurrence of the Aschoff body in the auricular appendage biopsy material have been grouped according to age. Early Aschoff bodies are much more frequent in the younger age group.

ess is progressive and ends in ultimate collagenization. While the clinical diagnosis was always "rheumatic heart disease," the findings of the tissue biopsy in 81.2% of the cases could not substantiate this diagnosis. Instead, the picture was one of a healing or healed carditis. Such findings do not negate the clinical diagnosis but rather offer tentative support, inasmuch as fibrosis and scarring represent the end-result of the primary collagen injury.

Failure to demonstrate definitive rheumatic criteria in postmortem hearts showing single valvular or multivalvular disease poses several problems. Either the involved tissues show the healed or healing stages of rheumatic carditis or the nonspecific changes secondary to unknown causes. Can these hearts still be referred to as rheumatic simply on the basis of healed valvular changes? The end-results of carditis and of valvulitis, regardless of cause, must be identical, owing to the limited capacity of the cardiac structures to react to noxious stimuli. If one accepts the thesis that calcific aortic stenosis may be nonrheumatic or rheumatic in origin, one might extend such reasoning to the consideration of mitral stenosis.

The constant association of the Aschoff body with rheumatic carditis acknowledges a specific response to a highly characteristic stimulus. Recent observations concerning the prophylaxis of rheumatic fever and epidemiologic studies have proved the efficacy of penicillin in removing the beta-hemolytic Streptococcus from the body. Serological evidence has added to the mass of indirect data which indicate that the Streptococcus occupies an important role in the pathogenesis of rheumatic fever. While other exciting factors are considered, the bulk of our present information implies that the Streptococcus is the key which triggers the reaction. Some investigators have recently commented on the isolation of the Streptococcus ‡ from the hearts of rheumatic cases, and others have demonstrated that live streptococci must be present in the active rheumatic state.[30]

These cumulative observations lead to a consideration of the Streptococcus and the Aschoff body per se. Although it is possible that a direct relation exists between the two, this has not been proved as yet. The same consideration applies to a relation between streptococcal injury and fibrinoid alteration of the ground substance. Fibrinoid alteration of the ground substance is believed to be different in the various collagen dis-

‡ References 28 and 29.

eases.§ Thus, if a variety of fibrinoids exist, it is not unreasonable to consider its presence in the acute rheumatic state as a manifestation of streptococcal activity. The possibility that these concepts are valid would help explain many problems in the pathogenesis of the disease, and future investigations should be directed along these lines.

The host factor in rheumatic fever has assumed increasing prominence. The studies of Wilson [33] have indicated that genetic factors are responsible for the predilection of the disease for certain persons. Selye [34] implies that the predisposition to rheumatic fever resides in a distorted endocrine balance. The well-known high frequency of the disease in childhood, when physiological activity of the endocrines is at a low level, is further advanced by these workers. Thus, Selye considers the development of rheumatic fever as a "stress" disease.[35] On the other hand, McKusick [36] has implied that a number of morphologic abnormalities of the cardiovascular system may be underlined by a congenital defect of mesenchymal elements which may become apparent later in life. Hereditary susceptibility to the rheumatic state could be explained on the basis of a more or less localized inborn or acquired aberration of collagen fibers which manifests itself by hyperirritability to environmental stimuli, of streptococcal or of different nature. Since the host factor constitutes a variable of large proportion and since rheumatic fever may result from a variety of agents, it is not surprising that the manifestations of the disease range from clear-cut to vague and bizarre. Such a state of affairs tends to limit suitability of calling the process a disease but suggests rather that it be termed a syndrome. If the idea of a rheumatic syndrome is maintained, then the concept of typical and atypical cases can be disregarded.

SUMMARY AND CONCLUSIONS

These observations are based on the analysis of 400 biopsy specimens from left auricular appendages removed during the course of cardiac surgery. In all cases the preoperative diagnosis was "mitral valvular disease" of rheumatic origin. Extensive clinical laboratory evaluation had established these cases as inactive prior to surgery.

The pathological findings lead to a classification of the cardiac biopsy specimens into two main categories: active rheumatic carditis and healed or healing rheumatic carditis. The rheumatic process was considered active in the presence of an exudative type of inflammatory reaction in the Aschoff body, in the endocardium or in the myocardium (independently from mural thrombosis), alteration of collagen fibers, and presence of "fibrinoid" in the ground substance. Damage of myofibers was a concurrent change, and, since these unequivocal signs of acute inflammation were in every instance accompanied by endocardial and myocardial fibrosis, this combination of recent and old lesions was interpreted as representing a condition of chronic rheumatic carditis with reactivation. Of the 400 appendages examined, only 8 (2%) fell into this category. In the absence of these changes and in the presence of senescent Aschoff bodies and of endocardial or myocardial fibrosis, the rheumatic carditis was then considered in a healed or healing stage. Of the 392 cases in this category, 67 (16.8%) showed Aschoff bodies.

It is indicated that the Aschoff body per se is not a reliable exponent of rheumatic activity. In the life cycle of rheumatic carditis, the earliest injury is a fibrinoid alteration of the ground substance. Exudative inflammatory reaction, myofiber necrosis, swelling, and fragmentation of collagen fibers are associated features of the early phases of the rheumatic process.

The transition to the chronic phase is characterized by the appearance of senescent changes within the Aschoff body and progressive endocardial and myocardial fibrosis.

Reactivation of the rheumatic process is accompanied by the appearance of fresh lesions, as shown by the association in the active carditis group of old and recent alterations.

Except for elevation of the erythrocyte sedimentation rate, which correlated with

§ References 31 and 32.

the pathological classification, no other clinical or laboratory indication of preoperative activity was found in any of the 400 cases, includng the 8 who had dsplayed unequivocal evidence of acute carditis in the biopsy specimen.

The postoperative course also failed to reveal in any of the cases signs, symptoms, or laboratory data suggesting recrudescence of the rheumatic process, and histologically active patients did not show any increase in operative mortality or morbidity as compared with microscopically inactive patients.

It is suggested that the discrepancies between the morphological and clinical manifestations of rheumatic activity can be due to the widespread use of potent therapeutic agents (steroid hormones, antibiotics). These agents may alter the individual response with little effect on the underlying disease process.

In 22 patients who died shortly after surgery, the findings in the biopsy specimens agreed with those observed in other portions of the heart.

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Studies in Rheumatic Fever

II. Origin of Cardiac Giant Cells

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Shortly after the structure of the Aschoff body was elucidated, a controversy arose concerning the origin of the multinucleated cells which characterize the lesion. In 1906 Aschoff [1] reported additional studies and believed that these large cells originated from mesenchymal periadventitial cells. Saigo [2] argued that the giant cells in the Aschoff nodule could be traced from cardiac muscle. This concept was further advanced by Whitman and Eastlake,[3] who postulated that Aschoff bodies could develop from microinfarcts of the myocardium. Numerous investigators have negated the myofiber origin of the Aschoff giant cell on the basis of its cytology and location. However, the most recent advocate of the myofiber theory was Murphy.[4] His observations of human postmortem material and experimental lesions induced in rabbits led him to conclude that the Aschoff giant cell represented the sarcoplasm of damaged myofibers, proliferated muscle cell nuclei, and syncytial multinucleated cell masses beneath the sarcolemma.

The purpose of this paper is to present additional evidence concerning the evolution of the classical Aschoff cell. In addition, it will be shown that another type of giant cell can develop in cardiac tissue.

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MATERIALS AND METHODS

The tissues studied were obtained from biopsy materials of the left auricular appendage removed during mitral commissurotomy and postmortem hearts. A total of 400 appendages was evaluated, including 100 by special stains. Thirty postmortem cases were analyzed, including acute rheumatic pancarditis (1), polyarteritis nodosa (1), lupus erythematosus disseminatus (3), generalized scleroderma (1), generalized sarcoidosis (1), giant-cell myocarditis (1), and chronic rheumatic valvulitis and myocarditis (22). Sections of the hearts were taken according to Gross and Ehrlich.[5]

OBSERVATIONS

The classical giant cell of the Aschoff body body was readily identified by its ragged-edged, often ill-defined cytoplasmic border containing basophilic cytoplasm with one or more nuclei. The nuclei frequently showed an "owl-eyed" appearance or were pyknotic. Figure 1 shows the formation of a typical rheumatic giant cell in an area of extensive myocarditis from a case of fatal rheumatic pancarditis. Note the numerous mononuclear cells having the appearance of Anitschkow myocytes. These cells appear to be fusing to form the multinucleated Aschoff giant cell. Figures 2 and 3 demonstrate Aschoff nodules in the myocardium in areas of myofiber degeneration. The giant cells have scalloped cytoplasmic borders, basophilic cytoplasm, and characteristic nuclei. They are embedded in a matrix of injured collagen fibers in which lymphocytes are scattered. Dying myofibers possess an eosinophilic or muddy-red cytoplasm often showing distinct longitudinal fibrillation and cross striation in contrast to the rheumatic giant cells which display none of these features. Figure 4 shows an Aschoff body in the left auricular subendocardium separated and removed from the myocardium

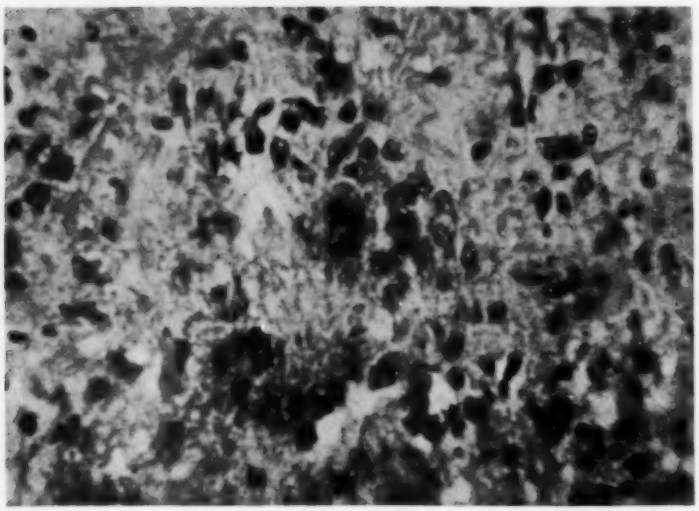


Fig. 1.—Focal areas of myocardial necrosis showing typical Anitschkow cells with "owl-eye" nuclei. Near the center of the field an Aschoff giant cell has formed with similar nuclear characteristics. Hematoxylin and eosin; reduced about ⅔ from mag. × 360.

by a dense collagen network. All of the Aschoff bodies observed in the appendages were located in the endocardium or subendocardial connective tissue.

Figure 5 illustrates giant cells in the myocardium in an area of myofiber degeneration. The section comes from the left ventricle of a heart demonstrating rheumatic valvulitis and scattered Aschoff bodies. The biopsy specimen of the auricular appendage removed during surgery had shown scattered senescent Aschoff bodies in the endocardium. A high-

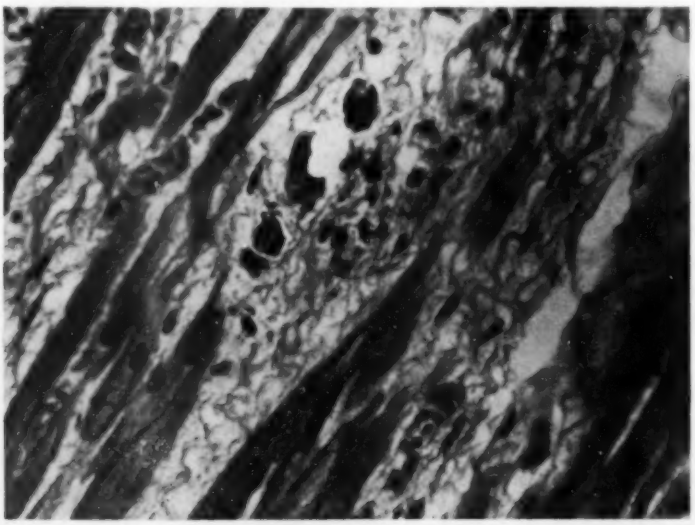


Fig. 2.—Two Aschoff giant cells present in an area of myofiber degeneration. Note the irregular cytoplasmic border and the nuclear detail. Collagen fibers are fragmented and granular. Hematoxylin and eosin; reduced about ⅓ from mag. × 360.

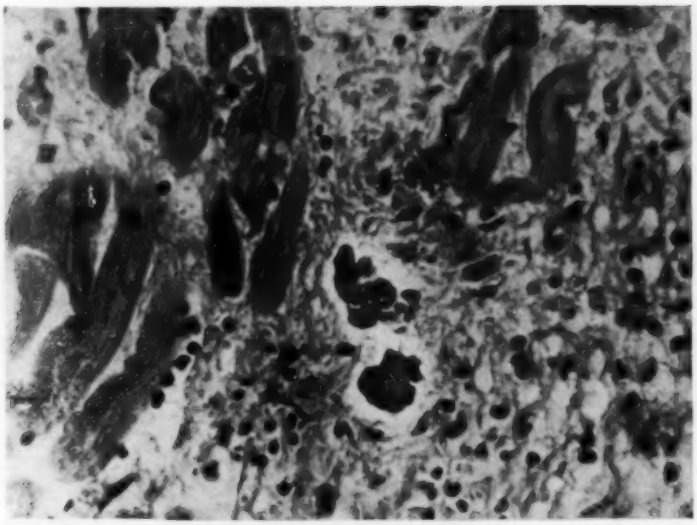
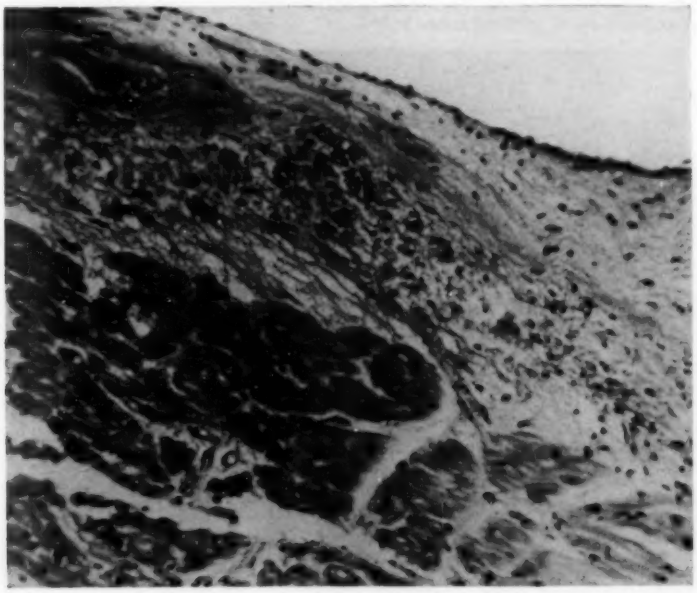


Fig. 3.—Typical rheumatic giant cells are present in an Aschoff body. The giant cells reveal the same properties as those in Figure 2. Hematoxylin and eosin; reduced about ⅓ from mag. × 360.

power view of the giant cells of Figure 5 reveals a cell having a definite border, eosinophilic cytoplasm, and peripheral, pyknotic hyperchromatic nuclei (Fig. 6). Similar cells were observed in the myocardium of four auricular appendages surgically removed from patients with mitral disease. In these instances there was myofiber damage with apparent increase in interstitial connective tissue adjacent to the giant cells.

Fig. 4.—Typical Aschoff body in the subendocardium of an auricular appendage. Definite separation, by connective tissue elements, between the lesion and the adjacent myocardium. Hematoxylin and eosin; reduced about ¼ from mag. × 175.



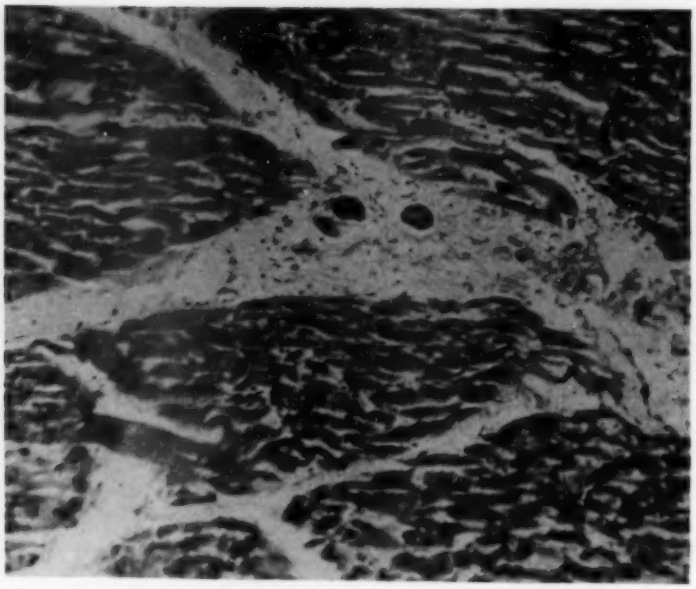
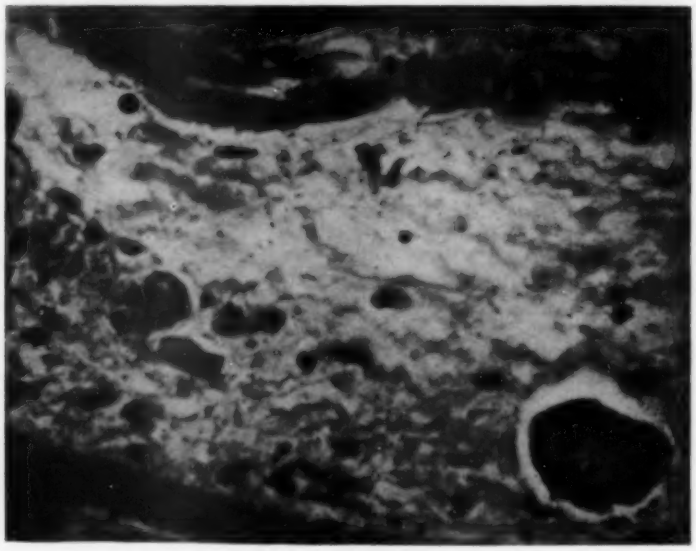


Fig. 5.—Cardiac giant cells of myogenic origin in an area of myofiber degeneration. Note the sharp cytoplasmic border and the rounded contours of the cells. Hematoxylin and eosin; reduced about ¼ from mag. × 175.

The accompanying Table illustrates the distinctive characteristics of the Aschoff body giant cell and the nonrheumatic giant cell of myogenic origin. The repeated finding of Aschoff bodies in the mural endocardium of the auricular appendages or elsewhere in the heart, the valve substance, and pericardium is a strong point against the myogenic origin

Fig. 6.—High-power view of a nonrheumatic giant cell revealing the peripheral distribution of hyperchromatic nuclei. Note the degenerated myofibers adjacent to the cell. Hematoxylin and eosin; reduced about ¼ from mag. × 360.



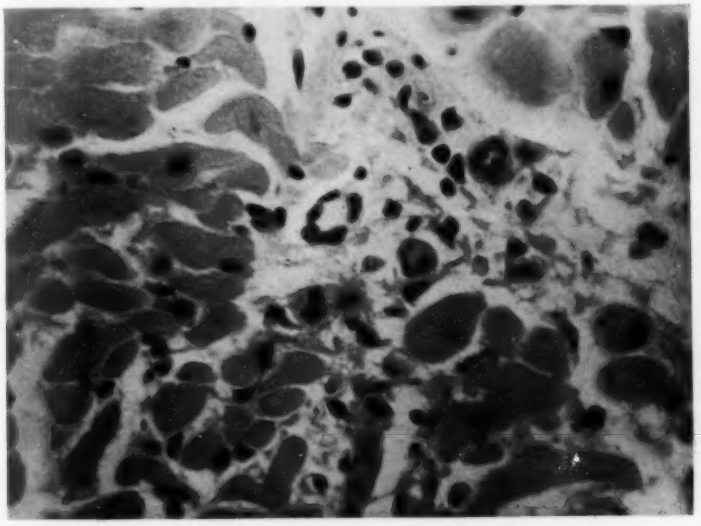
Microscopic Characteristics of Rheumatic and Nonrheumatic (Myogenic) Giant Cells

	Aschoff Giant Cell	Anitschkow Myocyte	Myogenic (Nonrheumatic) Giant Cell
Cell border	Ragged, ill-defined	Vague, ill-defined	Sharp, clear
Cytoplasm	Basophilic	Basophilic	Eosinophilic
Nucleus	Vesicular, "owl-eyed" appearance	Vesicular, "owl-eyed" appearance	Dense, hyperchromatic
Nucleolus	Often bar-shaped	Often bar-shaped	Not seen
Number of nuclei	1 to 4	1	4 to 8
Location of nuclei	Near center of cell	Center of cell	Periphery or irregularly scattered
Striations	Absent	Absent	May be present
Location of cell	Endocardium, myocardium, pericardium	Endocardium, myocardium, pericardium	Myocardium
Toluidine blue	± to + metachromasia	.....	.....
Ritter-Oleson	Dark blue	.....	Light, pink-blue
Phosphotungstic acid hematoxylin	No striations	No striations	Striations may be present

of this lesion. An evolutionary relationship is suggested between the Aschoff giant cell and the Anitschkow myocyte, which is now considered a special type of cardiac histiocyte (Fig. 1). The constant association of the nonrheumatic giant cells (Fig. 5) with myofiber degeneration and failure to detect such cells in areas free of myocardium serves to confirm an origin from damaged myocardium.

If the nonrheumatic myogenic giant cells were interpreted correctly, then they should be capable of demonstration in nonrheumatic heart disease associated with myofiber degeneration. Figure 7 is from a case of giant-cell myocarditis showing the presence of giant cells in the midst of a focal area of myofiber degeneration. A similar type cell was noted in the myocardium from a case of polyarteritis nodosa (Fig. 8). Here the nuclei are grouped together near one pole resembling the myogenic giant cells noted by Ruebner.[8] Figure 9 demonstrates the giant cell characteristic of Boeck's sarcoid from the myocardium of a case of generalized sarcoidosis. The cells are identified by the eosinophilic, often vacuolated, cytoplasm frequently containing proteolipid inclusions such as asteroid bodies. Figure 10 is a graphic representation of the cardiac giant cells and their possible relationships.

Fig. 7.—Cardiac giant cells of myogenic origin in giant-cell myocarditis. Peripheral distribution of the nuclei. Note the resemblance to Figure 6. Hematoxylin and eosin; reduced about ⅓ from mag. × 360.



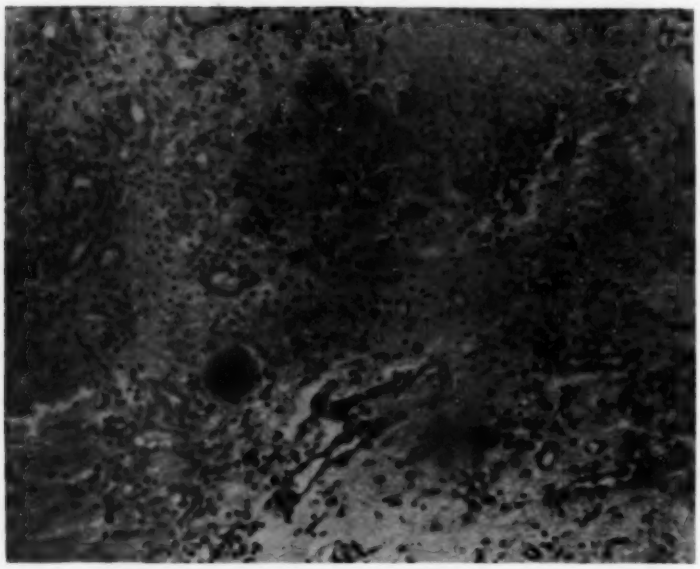
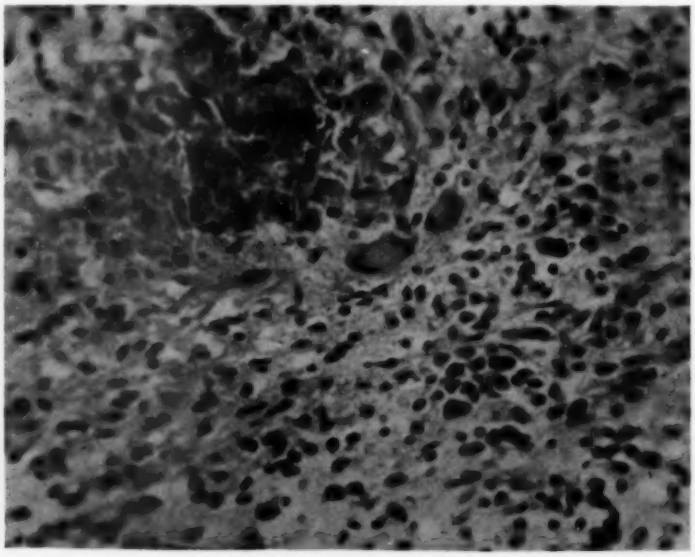


Fig. 8.—Area of acute myocardial necrosis in a case of polyarteritis nodosa. Giant cells are found in the midst of the inflammatory cells. Nuclei are grouped toward one pole of the cell. Hematoxylin and eosin; reduced about ¼ from mag. × 175.

COMMENT

The Aschoff bodies noted in the biopsy specimens of the auricular appendage were almost always in the endocardium and effectively separated from the adjacent myocardium by dense collagen fibers. Classically, the myocardial Aschoff bodies have been described as occurring in the interstitial con-

Fig. 9.—Typical foreign-body-type giant cells from a case of sarcoidosis. Area of acute myocardial necrosis present infiltrated by inflammatory cells. Hematoxylin and eosin; reduced about ¼ from mag. × 175.



nective tissue between bundles of myofibers. Finally, Aschoff bodies have been observed in the valves and pericardium. It is difficult to believe that the histopathogenesis of the Aschoff nodule is different for one or all sites of predilection. Murphy [4] is convinced from his studies that the myocardial Aschoff body develops from injured myofibers. He explains that the failure to demonstrate such an evolution in all instances is due to complete destruction of the myofibers, leaving no evident residua. In addition, Murphy postulates that myocardial Aschoff bodies can develop without evidence of primary injury to insterstitial collagen.

heart in 17 cases of rheumatic carditis and observed that the fibrinoid alteration of the interstitial connective tissue involved the sarcolemma of the muscle fibers also with resulting damage to these fibers. He further noticed giant cells of myogenic origin and contrasted them to the Aschoff giant cells. In agreement with the present study, Ruebner concluded that the Aschoff cells were derived from connective tissue cells, whereas the myogenic giant cells represented the limited regenerative ability of the myocardium.

Recognition of the myogenic giant cell as a separate and distinct entity is imperative

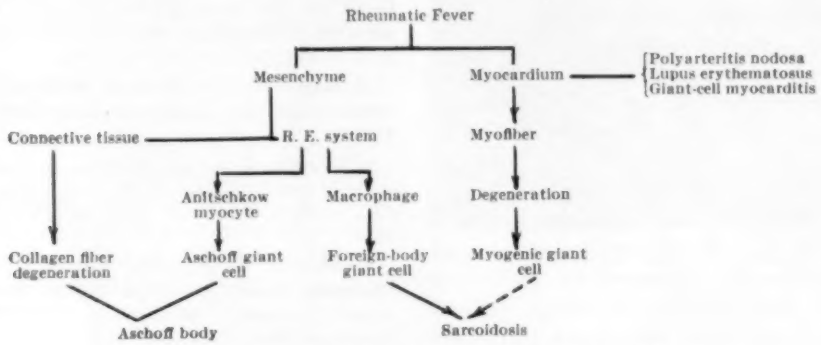


Fig. 10.—Histogenesis of the various cardiac giant cells.

The unequivocal demonstration of Aschoff bodies in sites utterly devoid of cardiac muscle casts doubt on the myofiber theory.[7] Similarly, the striking relationship between the Aschoff giant cell and mesenchymal elements peculiar to the heart tend to further negate such a concept. By use of special staining techniques we have been able to demonstrate abnormalities of the collagen fibers within and around all the mature Aschoff bodies encountered in this study.[8] Our observations confirm the opinion held by the majority of workers, namely, that the collagen fiber and ground substance changes are primary and precede the formation of the Aschoff cells which are exponents of a mesenchymal reaction.

Ruebner [6] has recently studied the relationship between muscle damage and the Aschoff cell in rheumatic carditis. He reviewed the

in the study of cardiac pathology. Figure 10 schematically demonstrates our concepts regarding the derivation of the various cardiac giant cells. Rheumatic fever strikes the cardiac mesenchyme and myocardium simultaneously, eliciting specific and nonspecific responses. The demonstration of myogenic giant cells in disseminated lupus erythematosus, polyarteritis nodosa, and giant-cell myocarditis indicates how the mistaken diagnosis of "Aschoff body" could be made in these diseases, unless it is understood that this type of giant cell is nonspecific of the rheumatic state.

Mention is made of the differences in histochemical properties between the Aschoff giant cell and the myogenic variety. The cytoplasm of the Aschoff cells demonstrates metachromasia and gives a moderately intense blue color by the Ritter-Oleson tech-

nique. Contrasted to these findings, the myogenic cell is nonmetachromatic and gives a pink-blue appearance when stained as previously mentioned. These findings would imply that the cytoplasm of the Aschoff cell is richer in acid mucopolysaccharides than the myogenic cell. Detailed histochemical analysis of the developing Aschoff body is the subject of another report.[8]

A consideration of all the available evidence leads to the inevitable conclusion that the Aschoff giant cells are manifestations of primary injury to the cardiac connective tissue. The immediate response to the damaged collagen fibers and altered ground substance is the mobilization of the reticuloendothelial system. In this instance a specific element of the mesenchyme, the Anitschkow myocyte, appears to be ultimately converted into the classic Aschoff cell. While the Anitschkow cell was believed to be related to the myofibers, it is a cardiac histiocyte characteristic of the heart and apparently of direct importance in rheumatic carditis.[1] Thus the Aschoff cells are the specific manifestations of the response of the cardiac connective tissue to rheumatic injury. As indicated in this study and others, myocardial damage is also a feature of rheumatic carditis. Consequently giant cells may develop from sarcolemma nuclei in an attempt at muscle regeneration. Thus any process capable of eliciting myofiber injury may provoke this type of giant-cell response.

SUMMARY

During a study of 400 biopsy specimens of left auricular appendages and 30 hearts, two types of cardiac giant cells were observed. The giant cell specific for rheumatic fever was the major component of Aschoff bodies in the myocardium as well as in areas devoid of muscle. A second type of giant cell appeared to develop from damaged myofibers and was noted in cases of rheumatic fever, giant-cell myocarditis, polyarteritis nodosa, and lupus erythematosus. The Aschoff giant cell is derived from the reticuloendothelial system as a specific response to primary connective tissue injury. The myogenic type of giant cell may develop in any area of myofiber degeneration as a limited attempt at regeneration and is a nonspecific response.

Miss S. H. Shapiro prepared the histological material.

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Lymphatic Cyst of Transverse Colon

Report of a Case Radiographically Simulating a Neoplastic Polyp

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D. B. CLAUDON, M.D.
and
R. W. BYRNE, M.D., Milwaukee

Roentgenological examination of the lower gastrointestinal tract in the course of annual physical examinations of persons over 40 years of age has been accepted as valuable in the detection of early asymptomatic lesions. The usual diseases identified by such means are diverticulosis, polyposis, and carcinoma. In a recent case with radiographic changes interpreted as those of a solitary neoplastic mucosal polyp of the transverse colon, laparotomy revealed an unusual basis for the observations in the form of a cystic submucosal lymphangioma. Because of the interesting features incident to diagnosis and treatment of the case, a brief report of the clinical and pathological data has been prepared.

Submitted for publication July 8, 1955.

From the Departments of Laboratory Medicine and Radiology, of Columbia Hospital.

Fig. 1.—Double contrast (air-barium) study shows rounded mass of tumor lying on inferior inner surface of transverse colon. Note meteor-tail-like folds demonstrating expansile pedicle radiating proximally from mass.

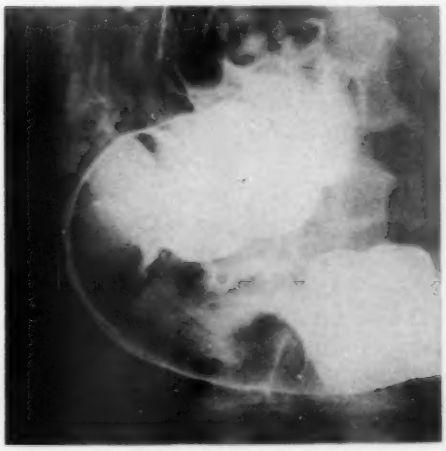


Fig. 2.—Magnified view of air-study shows pedicle to better advantage. Site of origin at sharp fold of colon along its superior surface can be identified.

REPORT OF A CASE

A 54-year-old newspaper reporter, was admitted to the Columbia Hospital in March, 1953, for resection of a polyp. The polyp had been discovered earlier in his transverse colon by barium enema examination as part of a routine annual health study. The patient had had no symptoms referable to his lower alimentary tract since a hemorrhoidectomy in 1951.

Routine physical examination of the colon by means of barium enema in 1951, prior to the hemorrhoidectomy, revealed no abnormalities.

In March, 1953, reexamination of the colon by barium enema, including routine preevacuation and postevacuation films and double-contrast air films, revealed the presence of a filling defect, thought to be caused by a polyp. The lesion was to the right of the midline in the transverse colon and was about 2 to 3 cm. in diameter. The examination also

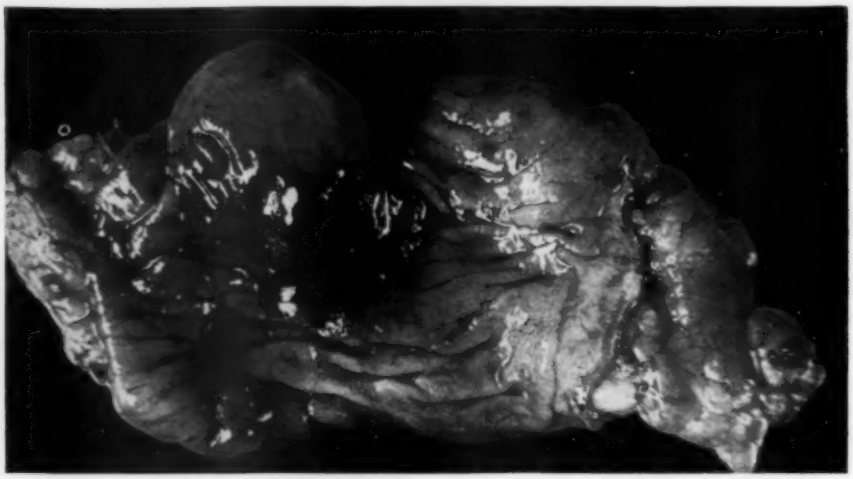


Fig. 3.—Opened surgical specimen shows the hemispherical mound on the anterior wall.

suggested that the polyp was possibly on a narrow stalk 3 to 4 cm. long, the base of which was proximal to the main mass (Figs. 1 and 2).

Palpation of the transverse colon at operation revealed the presence of a resilient, noncompressible, pedunculated mass within the lumen of the colon midway between the hepatic and splenic flexures. When the mass was isolated between the surgeon's fingers, the anterior wall of the colon bulged in a thinned area 1.2×1.5 cm. between tineae and it became apparent that the lesion was not a solid mucosal polyp, but a thin-walled cyst. A segment of the colon 6 cm. long was then resected, including an adequate cuff about the lesion.

PATHOLOGIC FINDINGS

When the surgical specimen was opened by longitudinal incision along the posterior-superior border, a hemispherical mound 2.5 cm. in diameter was seen in the anterior wall of the middle third (Fig. 3). The mucosa over the mass was normal. Traction on the mass resulted in the appearance of a short stalk or pedicle of lax mucosa and underlying tissues (Fig. 4). The stalk appeared attached to the area of the defect in the intestinal wall noted at operation.

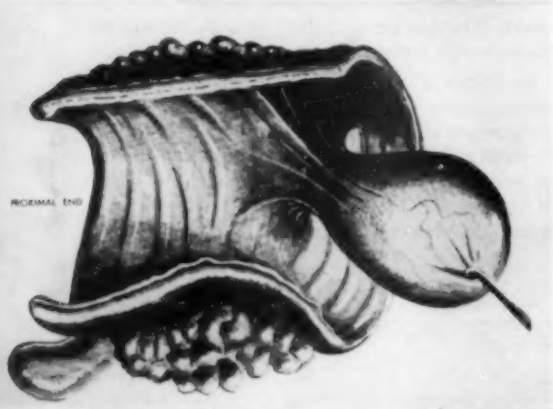


Fig. 4.—Traction on the mass results in the appearance of short stalk that was seen during the x-ray examination.

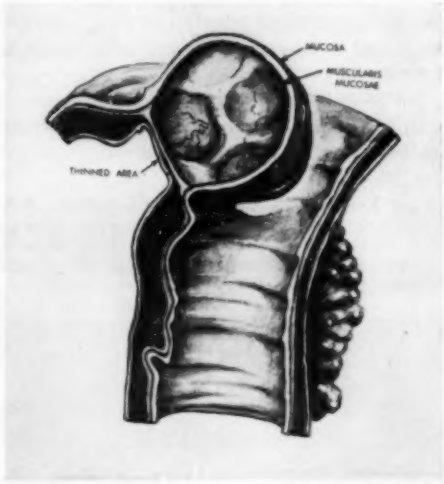


Fig. 5.—Longitudinal section of fixed specimen through polyp.

Incision of the mucosa exposed a unilocular, thin-walled submucosal cyst filled with clear amber fluid. The mucosa was of normal thickness and slid freely over the cyst (Fig. 5). When the specimen was placed in 10% formalin, a milky-white cloudiness developed

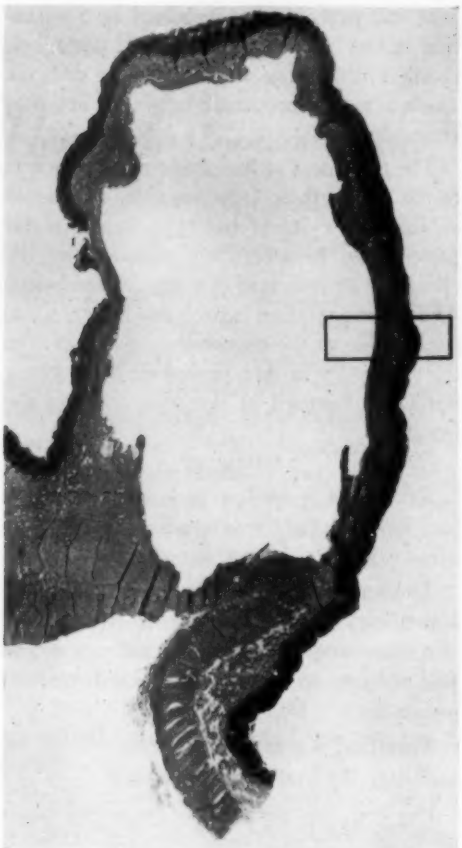


Fig. 6.—Microscopic section of the submucosal cyst. Hematoxylin and eosin; reduced about ⅙ from mag. × 8.

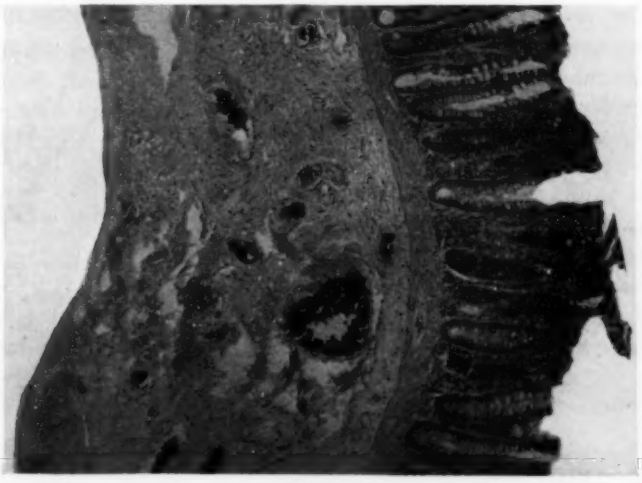
in the cyst fluid. Unfortunately, analysis of the fluid was not made prior to fixation.

Histological preparations were stained with hematoxylin and eosin, Verhoeff's elastic tissue stain, and Gomori's trichrome stain. The cyst was confined to the submucosa of the colon (Fig. 6). It was covered by normal mucosa and a somewhat thinned muscularis mucosae. The muscularis was atrophic, presumably due to pressure in the thin area of the anterior wall of the colon, but adjacent to this area the muscle was normal.

The cyst was lined by a well-defined, single layer of flattened endothelial cells; the cells rested upon the delicate areolar tissue of the submucosa, somewhat concentrated in places, apparently by pressure from the enlarging cyst (Fig. 7). There was no lymphoid tissue or evidence of inflammation in the wall or lumen of the specimen in any of a large number of sections. The submucosa beyond the cyst was likewise normal, and no connection could be demonstrated between the cyst and submucosal lymphatics. Neoplasia as evidenced by cellular proliferation was lacking.

The patient's postoperative course was uneventful. His convalescence was brief and complete recovery ensued.

Fig. 7.—Enlarged view of cyst wall (inset, Fig. 6); reduced slightly from mag. × 100.



COMMENT

Interest in this case stems from the fact that while radiographic examination detected the presence of an intestinal polyp in an asymptomatic patient, it led to an erroneous preoperative diagnosis. This mistake, of academic interest only, was also made by the surgeon when he palpated the lesion through the wall of the transverse colon and when he inspected the lesion after opening the intestinal wall. The redundancy of the mucosa and submucosa about the cyst was such that the polyp which it formed extended distally from its base within the lumen of the bowel and was indistinguishable from a pedunculated mucosal neoplasm. It was only after examination of the surgical specimen that the unusual nature of the polyp became evident.

In retrospect there appeared to be no radiological features which might aid in the future recognition of a similar case. Consideration of such a condition in the differential diagnosis of intestinal polyps would make for thoroughness, but the rarity of lymphatic cysts in this location renders accurate preoperative diagnosis unlikely.

From the standpoint of pathogenesis, considerable support can be found for the interpretation that the lesion represents a benign lymphatic neoplasm. Wegner [1] and, later, Gerster [2] have shown that cystic lymphangiomas may arise from cavernous lymphangiomas and may have cavernous lymphatic tissue in their walls. Cystic lymphangiomas seldom have connections with lymph channels, are usually solitary, and are not compressible. When found within the abdomen, the cysts oftenest have been within the mesentery and have contained chyle. Retroperitoneal cysts have been less common and have usually contained serous fluid. Rarest of all have been the submucosal cysts of the intestines. A majority of these have appeared in the small intestine and have contained chyle. The serous content of the cyst reported here can probably be explained by its location in the colon. The absence of pathologic changes in the tissues about the cyst does not speak against a neoplastic origin, as noted by Naumann.[3]

The treatment of lymphatic cysts varies, of course, with their location, size, etc. Small submucosal cysts of the type found in our patient can be effectively removed by excision, or by resection of a segment of bowel. The latter operation is probably better. Cysts comparable to the tremendous ones found in the mesentery or retroperitoneal tissues have not been observed in the submucosa of the intestines.

SUMMARY

A case of a benign pedunculated cystic lymphangioma in the submucosa of the transverse colon of a 54-year-old man is reported.

The tumor was found on radiographic examination of the gastrointestinal tract during a routine annual physical examination and was thought to be a pedunculated mucosal neoplasm.

Resection of a segment of transverse colon including the cyst resulted in cure.

This case was presented at the annual cancer clinic of Penrose Cancer Hospital, Colorado Springs, Colo., on Oct. 9, 1954, where the interpretation given above was accepted.

Dr. A. C. Gorder granted permission to publish this case.

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Neuroblastomas of the Nasal Fossa

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Neurogenic tumors of the nasal fossa, which are rare, may be classified as developmental and neoplastic. Examples of the former include the so-called nasal gliomas and encephaloceles or basal hernias. Morphologically these are characterized by a disorderly arrangement of glial tissue with interspersed bands of connective tissue. Bizarre astrocytes, ganglion cells, and spaces lined by ependymal cells are occasionally encountered, but mitoses are rare. Except for the two cases reported by Black and Smith,[1] in which there was recurrence following apparently complete removal, the fact that autonomous growth has not been observed indicates the non-neoplastic nature of such lesions.

Such neurogenic neoplasms as neurofibroma, neurilemmoma, and ganglioneuroma have been infrequently noted within the nasal fossa.* Their morphologic appearance and biological behavior appear to be similar to that observed for these tumors in their more familiar sites.

Of particular interest are those neoplasms of the nasal fossa characterized by an undifferentiated neuroectodermal structure identical to that observed in tumors of the adrenal medulla and the ganglia of the sympathetic nervous system. Berger, Luc, and Richard [4] in 1924 were the first to describe such a nasal lesion, which they encountered in a 50-year-old man. Because of the presence of neuroectodermal rosettes, glial fibrils, and the possibility that the tumor arose from the sensory cells of the olfactory mucosa, the name *esthésio-neuro-épithéliome olfactif* appeared appropriate. Two years later Berger and Coutard [5] encountered a similar tumor in this location differing from the former only in the absence of rosettes. They designated this more differentiated variant an "esthésio-neurocytome olfactif." Until 1951, when Schall and Lineback [6] reported 3 cases and Seaman [7] 1 in the American literature, there were only 14 examples of such a lesion recorded, all in the European literature. During the next four years 9 additional cases have been reported in this country, bringing the total number to 27.

The intranasal neuroblastomas do not produce symptoms which might be considered specific. Obstruction and epistaxis, as with other lesions in this region, are most frequent. There is apparently no sex or age predilection. Examples have been observed in a 13-year-old boy [8] and a 79-year-old woman.† The majority of these tumors, unlike their adrenal or ganglionic prototypes, have been considered as relatively benign. Invasion of the facial bones with exophthalmos has been occasionally observed. Distant metastases have been considered an unusual event,[9] although cervical lymph node metastases were found eight years after diagnosis in the case reported by Seaman [7]; and McCormack and Harris [10] observed two patients who died with widespread metastases 18 and 24 months after recognition of their primary tumors.

The purpose of this report is to direct attention to this type of nasal lesion, which has been neglected in the pathological literature and until recently has received little attention in clinical publications in this coun-

Submitted for publication July 20, 1955.

From the Departments of Pathology, University of Pittsburgh and the Veterans Administration Hospital.

* References 2 and 3.

† Lenz, M., cited by Seaman.[7]

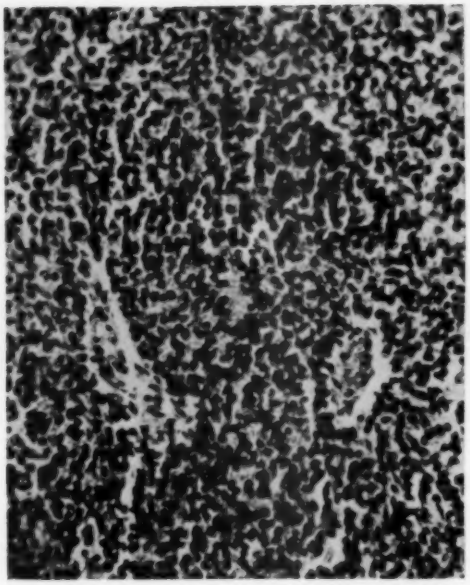


Fig. 1.—Section of nasal neuroblastoma demonstrating masses of round and ovoid cells with interspersed blood vessels. A pseudorosette is evident in the center. Reduced about ⅓ from mag. × 150.

try. Familiarity with this type of neoplasm should alleviate the difficulty occasionally encountered in the diagnosis of certain undifferentiated neoplasms of the nasal fossa. Since the majority of these tumors appear to be radiosensitive, their recognition is of practical value.

REPORT OF A CASE

A 31-year-old white man first experienced "stuffiness" of the nose in 1945, which was diagnosed as sinusitis. However, because of persistence of this symptom as well as an episode of epistaxis, he consulted another physician who informed him he had a nasal tumor. Biopsy was performed. Actual histological diagnosis was not rendered, although many competent pathologists examined sections of the lesion. The consensus was that the neoplasm was either a hemangiopericytoma, hemangioendothelioma, or undifferentiated carcinoma. In the following year and a half local excision was performed on three occasions, as well as x-ray therapy to the nasal fossa. The latter was moderately successful in controlling the local lesion and alleviating the nasal obstruction. However, a tumor nodule appeared in the hard palate, which was excised. Histologic diagnoses were similar to that recorded for the primary lesion. Local excision was inadquate on three occasions and radical resection of the hard palate, portion of the maxilla, and right zygomatic arch was performed. In addition, the patient experienced exophthalmos of the right eye and severe facial pain. X-ray examination of the facial bones revealed a destructive lesion in the right orbital bones.

The patient was hospitalized on numerous occasions because of pleural effusion due to pulmonary metastases as revealed by x-ray. On each occasion x-ray therapy resulted in a dramatic decrease in the size of these metastatic nodules.

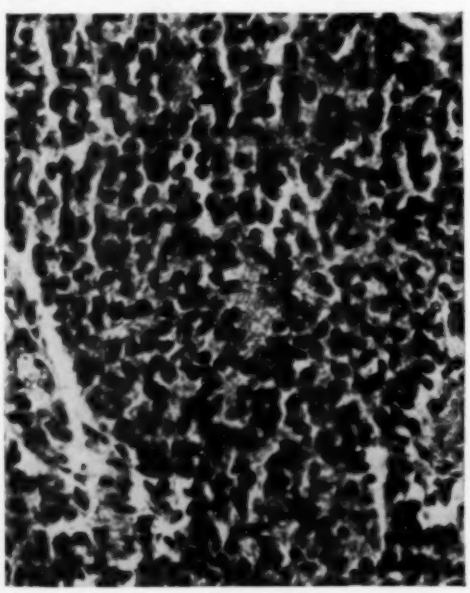
Physical examination at the time of his last hospital admission revealed marked emaciation. Exophthalmos of the right eye was present, accompanied by absence of the light reflex. The nasal septum was almost completely destroyed, as was most of the hard palate. Brownish-gray tumor tissue was observed projecting into the oral cavity through the bony defect in the hard palate.

The patient's general condition deteriorated and he died 10 years after the onset of symptoms and primary excision of the nasal tumor.

PATHOLOGICAL FINDINGS

Autopsy Findings.—The lungs weighed 2000 gm. Their surfaces were covered with dense adhesions and contained locules of fluid. Nodules of grayish-red, friable tumor

Fig. 2.—Higher magnification of field depicted in Figure 1 revealing cytologic detail and pseudorosette. Reduced about ⅓ from mag. × 300.



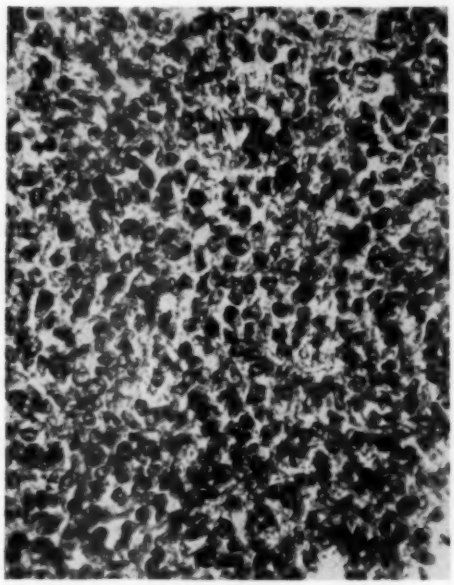
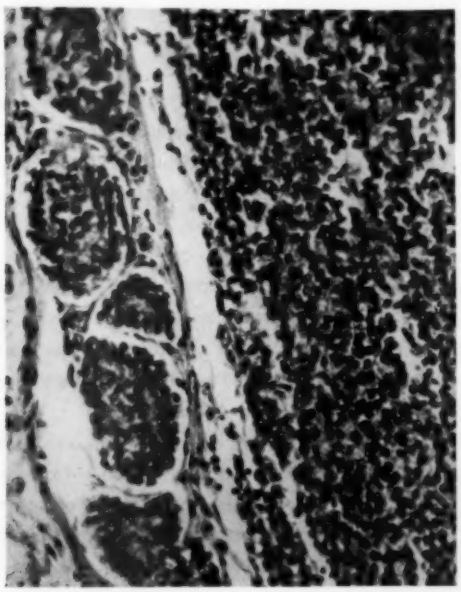


Fig. 3.—Section of pulmonary metastasis revealing pseudorosettes in endothelial-lined spaces. Reduced about ⅓ from mag. × 150.

tissue with necrotic centers varying from 3 mm. to 4 cm. were present throughout both lungs. Areas of fibrosis, edema, and bronchopneumonia were also present. The brain was displaced forward and upward owing to a tumor mass measuring 4 cm. in cross diameters which protruded through the base of the skull in the olfactory region.

Fig. 4.—Area from the primary lesion stained by the Masson technique revealing intercellular fibrils. Reduced about ⅓ from mag. × 300.



Microscopic Examination.—Sections and paraffin blocks of the original biopsy tissue from the nasal fossa were obtained for histological study. Tumor tissue obtained at necropsy was fixed in Zenker-acetic fluid and infiltrated with paraffin in the usual manner. Sections were stained with hematoxylin, eosin, and methylene blue; Masson's tri-

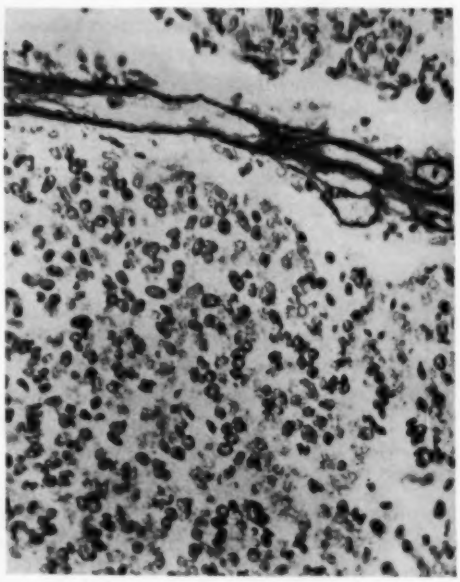


Fig. 5.—Section stained by the Wilder's reticulum method revealing lack of reticulum fibers about individual or groups of cells. Reduced about ⅓ from mag. × 400.

chrome method; phosphotungstic acid hematoxylin method of Mallory, and Wilder's reticulum technique. The morphological features of the pulmonary metastases and primary tumor were similar. All revealed a malignant neoplasm formed by large sheets and masses of uniformly round and occasionally ovoid cells, larger than a lymphocyte, lying in a network of fine fibrils. Pseudorosette formation was observed in areas (Figs. 1, 2, and 3). The cytoplasms of the tumor cells were, for the most part, indis-

tinct and when evident were scant. The nuclei were round and ovoid with finely divided chromatin and on occasion contained a distinct nucleolus. Mitotic figures were infrequently noted. Imperfect fibrous tissue trabeculae were distributed throughout the neoplasm and contained well-developed, distinct blood vessels. Tumor necrosis was also apparent. The fibrils observed in sections stained with hematoxylin, eosin, and methylene blue were more distinct when stained by the trichrome and phosphotungstic acid hematoxylin techniques (Fig. 4), appearing orange-red with the former and blue with the latter. The reticulum stain revealed preexisting reticulum fibers of the supporting tissue. There was no association of reticulum fibers with individual or groups of tumor cells (Fig. 5).

Diagnosis.—Nasal neuroblastoma with metastases to lungs and direct extension to the brain.

COMMENT

The terminology employed for these undifferentiated neuroblastic tumors of the nasal fossa has been similar to that for such tumors of the adrenal medulla and sympathetic ganglia. The value of designating these latter as neuroepitheliomas when well-formed rosettes of epithelial-like cells are present, neuroblastomas when characterized by pseudorosettes and abundant fibrils, or neurocytomas when rosettes or pseudorosettes are lacking has been justifiably criticized.[11] It is interesting to note that in some instances of these nasal tumors a correlation between the more poorly differentiated lesions (neuroepitheliomas and neuroblastomas) and clinical behavior has been obtained.[10] However, exceptions to this are evident in other reported cases. In addition, too few examples of these nasal tumors have been adequately followed to ascribe prognostic significance to the morphologic variants encountered. The lesion described in this report has been indicated as neuroblastoma not only because of its structural characteristics but also in the generic sense.

The difficulty in establishing the true nature of the nasal neoplasm in the case reported undoubtedly arose from a lack of familiarity with the possibility of such tumors being present in this site. Its confusion with vascular tumors or undifferentiated carcinoma has been previously noted.[12] The utilization of the Masson and phosphotungstic acid hematoxylin stains for the identification of the fibrillary components of these lesions aids in establishing their neuroblastic nature. In our experience, the former has been more useful for this purpose. The reticulum stain is also of value in the differentiation of this neoplasm from vascular tumors. Unlike the latter, reticulum is not evident about individual cells or cell groups, and otherwise occult vessels are not present. That neuroblastic tumors may arise within the nasal fossa is evident from the fact that both the epithelial and neural portions of the olfactory mucosa originate from the same neuroectodermal thickening, the olfactory placode.[13] Other possible sites of origin for such tumors which have been suggested are Jacobson's organ[6] and the sphenopalatine ganglion.[14]

Unlike similar lesions of the adrenal medulla or sympathetic ganglia, most examples of nasal neuroblastoma have pursued a relatively benign clinical course. The fact that the location of the latter results in early appearance of symptoms, as well as the opportunity for direct visualization and relatively easy extirpation, may have some influence on this difference in behavior. However, widespread metastases do occur with these lesions, as evidenced from this and previously reported cases.[10] Since such an event may be deferred for many years, prognosis must be based on longer periods of time than usually ascribed for other neuroblastomas.

The favorable effect of irradiation on these neoplasms is reemphasized from the observations of the dramatic decrease in size of some of the pulmonary metastases in the case presented.

SUMMARY

The literature concerning nasal neuroblastomas is briefly reviewed, and the clinical and morphological features of these neoplasms are presented.

A case is reported which illustrates certain features of these tumors. The difficulty encountered in the histological recognition of the true nature of this nasal tumor was probably due to a lack of familiarity with such an entity.

Although the rate of growth of the nasal neuroblastomas appears to be slower than that noted for similar lesions in their more familiar sites, such tumors are capable of widespread metastases and warrant a guarded prognosis. The patient presented in this report died 10 years after the recognition of his nasal tumor.

The radiosensitivity of these lesions is reemphasized by the dramatic reduction in size of pulmonary metastases following such treatment in the case presented.

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Cerebral Mucormycosis

Report of a Case

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and
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The Mucoraceae belong to the class Phycomycetes. They are the fluffy molds seen so commonly on decaying foodstuffs and horse dung. Spontaneous infections with the Mucoraceae have been reported frequently in animals, but they are not common in man. Invasion of the central nervous system is particularly rare, and only nine cases of cerebral mucormycosis have been reported in humans. * In each of these cases the infection occurred as a terminal event of a chronic debilitating illness.

We wish to report the occurrence of cerebral mucormycosis in a child suffering from chronic glomerulonephritis.

CASE REPORT

A 5-year-old girl was admitted to this hospital in April, 1950, with swelling of the feet and ankles. There was no history of a recent upper respiratory infection. Her tonsils and adenoids had been removed in April, 1949, for recurrent sore throats, but she had been otherwise healthy. On admission she was pale and moderately edematous; there was no hypertension. Massive proteinuria was present, but no red cells or casts were seen in the urine. A diagnosis of the nephrotic syndrome was made, and she was treated with two courses of oral cortisone. The edema subsided but the proteinuria persisted.

Three months later she was readmitted with ascites, and on this occasion she failed to respond to cortisone therapy. During the next three years she developed progressive anemia and progressive impairment of renal function. Ten days before death, at the age of 8 years, she was admitted with abdominal pain, vomiting, and pyrexia. She was slightly edematous, her blood pressure was 90/60, and the blood urea nitrogen was 68 mg./100 ml. Her condition deteriorated, and she lapsed into coma and died.

PATHOLOGY

Gross Findings.—An autopsy was performed one hour after death. The body was small and wasted. There was slight edema of the face and feet and a little excess fluid in the serous cavities. The kidneys were small and pale, and the capsules were adherent. The cortex was finely granular, and the corticomedullary junction was indistinct. The heart was normal, and the abdominal aorta showed a few small atheromatous plaques. The right adrenal was normal; the left adrenal contained a cyst, 2.5 cm. in diameter, which was filled with dark, altered blood. The remaining organs with the exception of the brain were normal.

The brain (1287 gm.) showed several interesting and unexpected findings. The left internal carotid and middle cerebral arteries were filled with recent red thrombus. The remaining vessels appeared normal. The left temporal lobe was dark red and soft, as though infarcted, and the whole of the left cerebral hemisphere was edematous. There was hemorrhage into the upper pons, left cerebral peduncle, left thalamus, caudate nucleus, and internal capsule. The right side of the brain appeared normal.

Microscopic Findings.—The thrombosis of the left internal carotid and middle cerebral arteries was confirmed. Thrombi were also present in the right internal carotid and the posterior communicating arteries. The basilar and vertebral arteries were normal. The arterial thrombi were composed of masses

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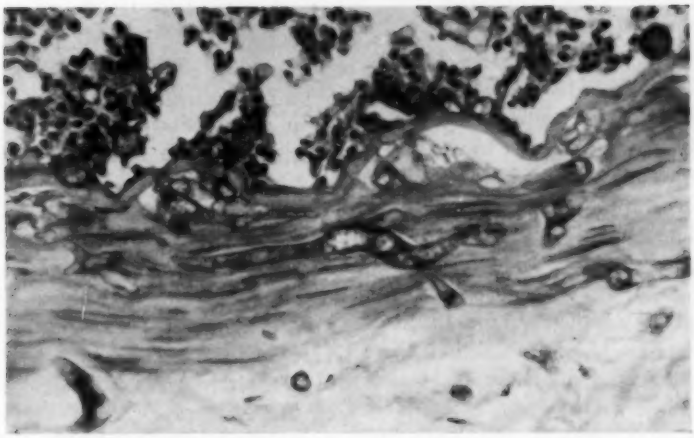

Fig. 1.—Left internal carotid artery; hyphae are seen in the wall of the vessel. Hematoxylin and eosin; reduced about ⅓ from mag. × 500.

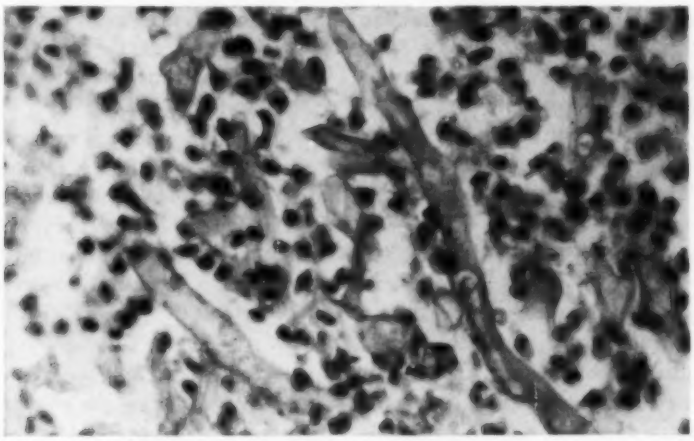

Fig. 2.—Nonseptate branched hyphae. Hematoxylin and eosin; reduced about ⅓ from mag. × 500.

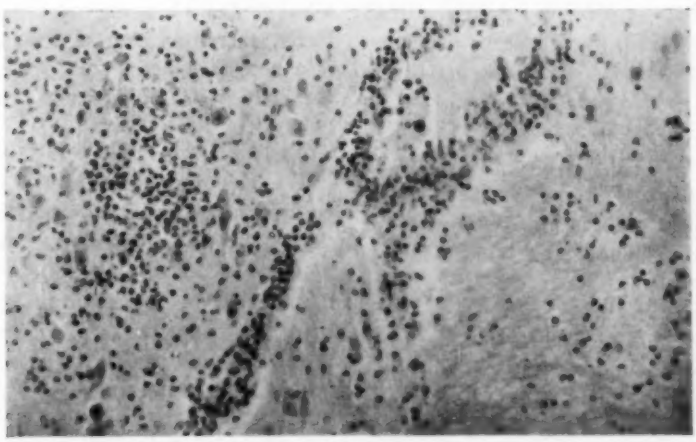

Fig. 3.—Polymorphonuclear cuffing of affected cerebral vessels. Hematoxylin and eosin; reduced about ⅓ from mag. × 150.

of polymorphonuclear leucocytes, red cells, and fibrin. There were also many filamentous hyphae, some of which were seen in cross section. Many of the filaments were seen in the vessel wall outside the internal elastic lamina (Fig. 1), and some were present in the basal subarachnoid space. The hyphae were large, nonseptate, and frequently branched (Fig. 2). They measured up to 0.2 mm. in length, and the average diameter was 12μ (range 6μ-15μ). In addition, there were a few spherical, eosinophilic solid bodies resembling spores which were distinct from the hyphae seen in cross section. They had an average diameter of 12μ.

Similar vascular lesions were present in the pons, midbrain, left thalamus and caudate nucleus, and the left frontal cortex. The right cerebral hemisphere showed no thromboses, but hyphae were present in a few of the vessels. A wide cuff of polymorphonuclear leucocytes surrounded the thrombosed vessels (Fig. 3), and the adjacent brain tissue showed a microglial reaction. No bacteria were demonstrated in the sections.

The kidneys showed the changes of chronic glomerulonephritis. There was hemorrhage into the left adrenal, but there was no inflammatory reaction and no organisms were seen. The following tissues were examined microscopically: thymus; trachea; thyroid; bronchi; lungs; heart; ascending, descending, and abdominal aorta; esophagus; stomach; small and large intestine; liver; spleen; pancreas; pituitary, and ovary. All these organs were normal.

COMMENT

Paltauf [1] (1885) described the first case of cerebral mucormycosis. The patient suffered from a long-standing gastrointestinal disturbance, and at autopsy there was ulceration of the intestine. Fungi were seen in the smears made from the ulcers, and they were also found in the lungs and brain. Only eight cases have been reported since that time. In all of the cases the diagnosis of mucormycosis was made on morphological grounds, the condition being recognized only at the time of microscopic examination of the tissues.

In our case we thought at the time of autopsy that the lesions of the brain were due to occlusion of the basal vessels. We did not, however, consider the possible infective nature of the condition, and no cultures were prepared. Microscopic examination confirmed the vascular occlusion and also demonstrated the presence of intravascular hyphae and spores. The morphology of these structures was identical with that of the Mucoraceae, and the intravascular growth has been reported in all the previous cases of cerebral mucormycosis. The presence of a brisk inflammatory reaction about the affected vessels, in the adjacent brain substance, and in the subarachnoid space indicated that this was an antemortem invasion by the fungus.

The portal of entry is unknown. No fungi were seen in the organs examined, but the nose and accessory air sinuses were not opened. Six of the previously reported cases † showed involvement of the orbit, and the infection appeared to have originated in the nose in the patient reported by Stratemeier.[5]

The condition has been associated with diabetes mellitus in six cases.‡ The infant reported by Martin and co-workers [6] was dehydrated and marasmic, and Kurrein [7] described the condition in an 8-year-old boy who died with acute nephritis. It seems likely, therefore, that a debilitating illness is a necessary precursor to the invasion of the body by this ubiquitous fungus.

SUMMARY

We have described a case of cerebral mucormycosis occurring as a terminal event in an 8-year-old girl suffering from chronic glomerulonephritis. Infarction of the brain consequent on vascular occlusion was the diagnosis at autopsy, and the true nature of the disease was recognized only when microscopic sections were examined. In this re-

† References 2, 3, 4, and 6.

‡ References 2 through 5.

spect our case resembles the nine previously reported cases. This is the fourth instance in which the condition has not been associated with diabetes mellitus. The feature common to all cases is a debilitating illness.

Dr. M. A. Cox granted permission to publish this case report and Dr. W. L. Donohue gave advice during its preparation.

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A Semiquantitative Dopa Reaction by Use of Frozen-Dried Skin

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Impetus was given to the study of melanogenesis by Bloch's [1] discovery that dihydroxyphenylalanine (dopa) specifically stains melanocytes. Not all workers, however, accepted Bloch's evidence that the reaction was due to a specific enzyme, dopa oxidase. In his critical review Meirowsky [2] points out that dopa is also oxidized by granules in neutrophiles, nerve fibrils, and certain bacteria and fungi. More recently Fitzpatrick and his co-workers [3] have presented evidence that the enzyme which catalyzes pigment formation in human skin is tyrosinase. Despite the modifications of Bloch's hypothesis, the dopa reaction remains the main technique for the study of melanogenesis.

The objection to present methods for histologic demonstration of the dopa reaction in the skin is the need for hardening tissues in formaldehyde before incubation in dopa. The unsatisfactory, thick preparations obtained by sectioning fresh-frozen skin led to the use of formalin, but the possibility of inactivation of the dopa oxidases has led investigators to advise hardening "briefly" (from 30 to 60 minutes) in "dilute formalin" (5% to 10%). It has been assumed that the activity of the enzyme is only negligibly altered by such treatment. Laidlaw [4] cites Bloch, Miescher, S. W. Becker Sr., and Peck to the effect that the dopa reaction is in no way impaired by two to three hours stay in 5% formalin. S. W. Becker Jr. and his co-workers,[6] however, observed that formalin fixation of epidermis separated from the dermis resulted in a loss of enzyme activity.

A comprehensive review and evaluation of the dopa reaction was published by Laidlaw [4] in 1932. In another study he and Blackberg [5] did much to simplify and standardize the technique originally described by Bloch.[1] The method first used by Bloch was to impregnate fresh skin with agar from which frozen sections were cut and incubated in dopa solution at 37 C for 24 hours. The disadvantages of thick preparations inherent in the cutting of fresh-frozen skin led him to modify his technique by hardening the tissue in 5% formalin for two to three hours before impregnation with agar.[4] In 1935 Becker, Praver, and Thatcher [7] improved the method by the use of paraffin, whereby better preparations were possible. They immersed the tissue in 10% formalin for one hour before incubating a fragment (3 to 5 mm.) in dopa for one hour and then in fresh substrate overnight. Following this, the tissue was fixed in Bouin's solution for 24 hours and finally impregnated with paraffin for sectioning.

The two purposes of this study were, first, to evaluate melanogenesis quantitatively and, second, to determine the degree of enzyme inhibition or destruction caused by brief hardening in formalin. The method for the dopa reaction reported in this study avoids the use of chemicals. To evaluate the effect of formalin on the enzymes, a comparison was

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made of tissues prepared by this method with those stained by Becker's technique.

MATERIALS AND METHODS

Fresh specimens of normal skin from 50 surgical patients chosen at random were obtained at the time of a surgical incision. The skin was obtained in late winter and, with five exceptions, from an area that had not been exposed to sunlight for over six months, mainly from the abdomen or breast away from the areola. The patients ranged in age from 19 to 61 years; 37 were white, and 13 were Negroes; 28 of the patients were women and 22 men. Each strip of skin was divided into five portions, approximately 5×5 mm. One of these was immediately prepared by the Altmann-Gersh method [8] of rapidly freezing at —150 to —160 C in isopentane cooled by liquid nitrogen, and then drying *in vacuo* at about —30 C. The other four specimens were placed in formalin, two in a 5% solution for one-half hour and one hour, respectively, and two in a 10% solution for the same periods. The formalin-hardened specimens were then prepared "en bloc" by the method of Becker, Praver, and Thatcher [7] previously described. After a number of preliminary trials, the one modification that was made in Becker's technique was to substitute fixation in 10% formalin overnight for Bouin's solution. This was done because the yellow background produced by Bouin's fixative proved to be less favorable for the detection of the brown-gray melanocytes of a slight dopa reaction than did the clear, unstained tissue.

The same concentration of water-clear dopa, kept under refrigeration (0.0065 M dopa in 0.1 M phosphate buffer, giving a pH of 7.4), was used throughout this study. A fresh solution was prepared as soon as the slightest color change was observed.

The tissue prepared by freeze-drying was imbedded in paraffin (M. P. 54 to 56 C). As in the formalin-hardened materials, four to six sections cut at 4μ and a similar number at 8μ were mounted on one slide. After removal of the paraffin with xylene, the preparation was incubated in dopa solution at 37.5 C. To determine the optimum time of incubation in the substrate, a preliminary study was made in which sections from one tissue were removed at frequent intervals for a period of seven hours. During this experiment color changes of the dopa solution were observed. It began to turn pink at two hours. Thereafter, it gradually changed color until at 3 hours 45 minutes it could best be described as very light gray-violet. This color change at 37.5 C in 3 hours 45 minutes was consistently present in all subsequent observations. Within 15 minutes after this time the color changed to a much darker violet-brown, and then rapidly to black at the end of 5 hours. The optimum period of incubation selected after numerous trials was 3 hours 45 minutes at room temperature. At this point the melanocytes had developed their most intense stain with little or no coloring of the adjacent epidermal cells or of the dermis. A longer period resulted in the absorption of the colored dopa by the surrounding tissue, especially by the nuclei of the epidermal cells which tended to obscure slight dopa reactions. The characteristic color of a positive dopa reaction obtained with this method varied from a gray-tinted cytoplasm containing a few black granules to an intensely black color. There was no brown tint of the cytoplasm characteristic of dopa reactions following formalin-hardening. The absence of brown color in melanocytes stained after freeze-drying was particularly helpful in tissues from Negro patients. After formalin fixation sections from highly pigmented areas may require very close study to distinguish melanocytes from melanin-containing basal cells.

After removal from the dopa solution the preparation was dehydrated in clear 95% alcohol (two minutes), followed by absolute alcohol (five minutes). It was then carefully cleared in three or more successive dishes of clean xylene until all of the black sediment (oxidized dopa) was removed. This procedure was essential for clean preparations. The sections were then mounted in balsam for histologic study.

RESULTS

A. Dopa Reactions in Frozen-Dried Tissues

The variability in thickness of sections cut at a given setting of the microtome was not an important factor in the comparison of the two techniques. The thickness of sections was estimated by means of the fine micrometer adjustment using a 10 × ocular and an oil-immersion objective. A total of 40 specimens from frozen-dried tissues cut at 4μ and the corresponding sections from formalin-hardened materials were compared. Of the formalin-hardened preparations, 32 were definitely thicker than the corresponding frozen-dried specimens and the remaining 8 pairs were approximately equal in thickness. The hard preparation resulting from freeze-drying could be cut more accurately than the soft, partially fixed tissue produced by brief immersion in formalin. Frozen-dried sections obtained at 4μ varied

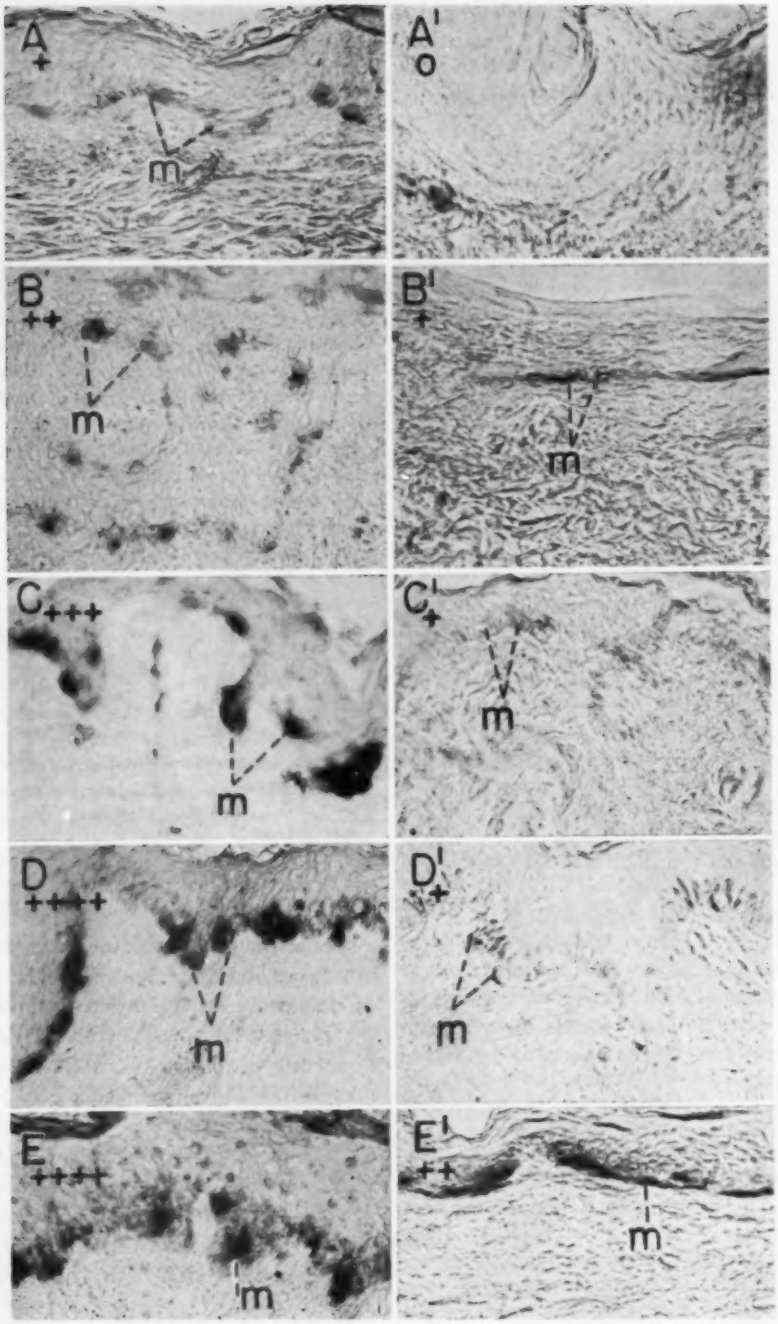

Fig. 1.—A comparison of the dopa reaction in five white patients whose skin was prepared by the technique described (*A*, *B*, *C*, *D*, and *E*) and by Becker's method (A^1, B^1, C^1, D^1, and E^1). Note the differences in the number of visible melanocytes (*m*) and their staining intensity in the frozen-dried preparations (left) compared with the corresponding formalin-hardened tissue (right). Note the diffusion of the dopa reaction (*m* in *E*). In frozen-dried tissues a wide variability in staining intensity of melanocytes is found in the same section (note *B* and *D*). The photography, development, and printing were made with a uniform technique, so that the variations in staining intensity of melanocytes are attributable primarily to the methods used; × 250.

no more than 20% from the desired thickness, while those from formalin-hardened materials were frequently 75% thicker than required. The difference in staining intensity of melanocytes by the two techniques was so marked that the greater thickness of formalin-hardened sections proved relatively unimportant.

As noted by Laidlaw,[4] the melanocytes may vary in their enzymatic activity. Therefore, it was not surprising to find cells in the same preparation with staining intensities varying from 1+ to 4+ (Fig. 1*B* and *D*). It was necessary to consider the reaction in the majority of the melanocytes for evaluating the over-all staining intensity of each

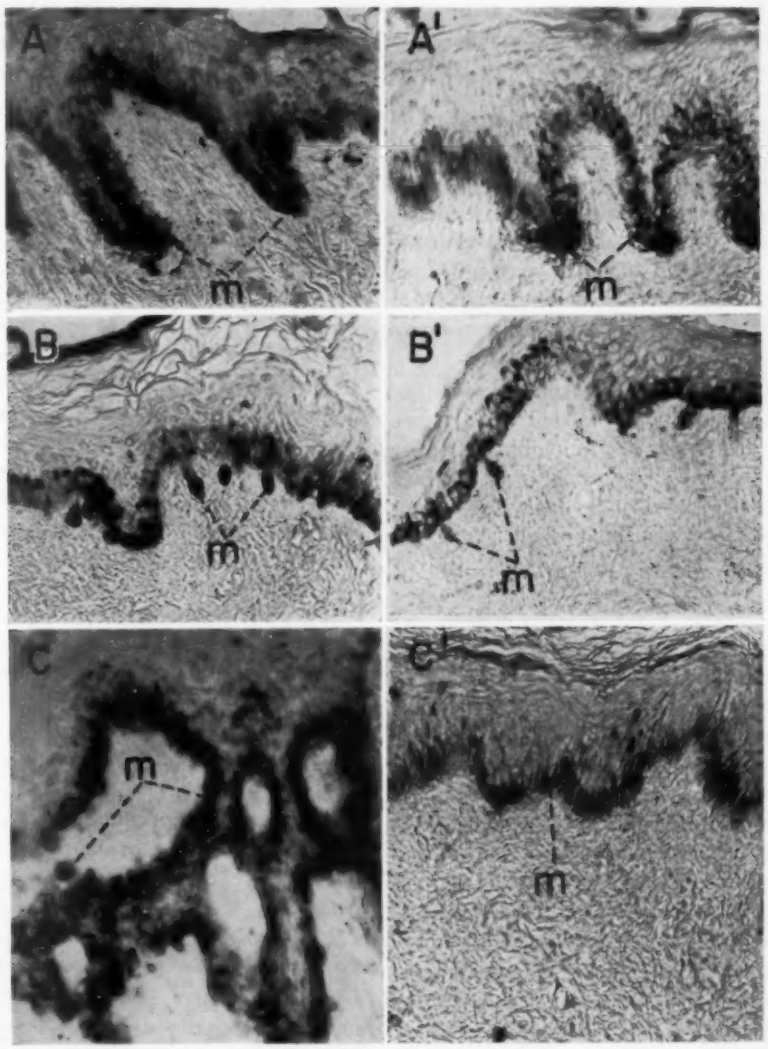

Fig. 2.—A comparison of the dopa reaction in three Negro patients whose skin was prepared by the technique described (left) (*A*, *B*, and *C*) and by Becker's method (right) (A^1, B^1, and C^1). The melanocytes in C^1 cannot be differentiated from the pigment-containing basal cells, except with a magnification of 400 or higher. At such magnification they appeared as angular, light-staining cells.

preparation. The following is a description of the criteria for grouping the preparations from frozen-dried tissues according to the staining intensity of most of their melanocytes. Our classification calls a 1+ (Fig. 1*A*) a reaction in which the majority of the melanocytes could be distinguished as cells with gray-stained cytoplasm, with or without a few fine black granules. In a 2+ reaction (Fig. 1*B*) the cells had many more black granules, but considerable gray-stained cytoplasm was still visible. A 3+ reaction (Fig. 1*C*) was characterized by such an abundance of black granules in the majority of melanocytes that little of the gray cytoplasm was visible; while in a 4+ reaction (Fig. 1*D* and *E*) the cytoplasm stained a solid black with no discrete granules discernible. The cells with the strongest reaction were comparable in staining intensity to neutrophiles. They were characteristic of Negroes and of dark-skinned persons.

The formalin-treated tissues prepared by Becker's method could be similarly grouped according to the degree of staining intensity of melanocytes. These, however, stained much less deeply than those in corresponding groups of frozen-dried preparations. If classified according to our method, the 1+ reactions in tissues prepared by Becker's method were so slight that the melanocytes were distinguishable only by close scrutiny at a high magnification (× 400). Unlike the dark, gray-stained melanocytes in the 1+ reactions of frozen-dried preparations, those in tissues prepared by Becker's method could not be seen at a lower magnification. This was due partly to the greater difficulty in detecting the brown-gray color of the melanocytes in the presence of cells filled with melanin. (Compare *B* with B^1 in Figure 1, and *C* with C^1 in Figure 2.)

An interesting finding in some of the preparations from frozen-dried tissues was the presence, around intensely stained melanocytes, of a gray zone containing fine black granules (Fig. 1*C* and *E*). The area surrounding these cells extended in the epidermis over a distance equivalent to the diameter of two to three basal cells. The staining intensity was greatest immediately adjacent to the melanocyte at the center of the zone and gradually decreased toward the periphery. This, we believe, represents dopa-positive granules within dendrites. When this zone was intensely stained, the morphologic details of the melanocyte were obscured, a phenomenon which occurred in some of the tissues with 3+ and 4+ reactions. In the specimens prepared by formalin-hardening and stained by Becker's method, such zones about melanocytes were less distinct or absent.

B. Comparison of the Dopa Reaction in Frozen-Dried and Formalin-Treated Tissues

The preparation with the strongest dopa reaction by Becker's method was selected from each group of four tissues treated with 5% and 10% formalin to be compared with that from the adjacent frozen-dried tissue. Only slight differences were observed in the four formalin-treated preparations of each tissue. These were related to the concentration of the fixative rather than to the time of exposure in it. In 30 of the tissues no difference could be discerned between the effect of 5% and 10% formalin. In 8 the reaction was slightly stronger after hardening in 10% formalin, while in 12 the 5% formalin produced definitely better results. The formalin-treated tissues, cut at 8μ and 4μ, prepared by Becker's method were compared with the corresponding adjacent specimens treated by the technique described here (Tables 1 and 2). The following distribution of the reactions were obtained in the 37 white patients (Table 1). In the frozen-dried tissues 4 were classified as 4+, 19 as 3+, 11 as 2+, 2 as 1+, and 1 as 0. Thus, 34 (92%) of the tissues from white persons in the 2+ or higher groupings and 36 of the 37 (97.3%) gave positive dopa reactions. In the corresponding tissue prepared by Becker's method 18 (about 50%) were classified as 1+, and 19 (about 50%) as 0. By this technique no tissue in this group gave more than a 1+ reaction. Not only the staining intensity but also the number of

visible melanocytes was less in the formalin-treated than in the corresponding frozen-dried tissues. (Compare the corresponding tissues in Figure 1 and *A* with *A*[1] in Figure 2.)

Specimens from the 13 Negro patients prepared with Becker's technique showed a marked reduction in the staining intensity of melanocytes compared with those treated by the technique described here (Fig. 2). In some specimens (Fig. 2*C*[1]) the pale-staining melanocytes could be seen only by careful search in thin preparations (4μ or less) under high magnification (× 400). This occurred in 5 of the 13 preparations. It is not unlikely that without very close scrutiny these would have been called negative dopa reactions.

In 8 of the 13, the reduction was in the number of visible melanocytes rather than in their staining intensity. In this group the melanocytes in the frozen-dried tissues were numerous and deeply stained, while those in the corresponding formalin-hardened preparations were few in number even though intensely stained. The proportion between the number of melanocytes in the frozen-dried and those in the corresponding formalin-hardened tissues was often greater than 10 to 1. (Compare *A* with *A*[1] in Figure 2.)

While this group of Negro subjects numbered only 13, the comparison of the results shown in Table 2 seems significant. In the frozen-dried tissues seven were classed as 4+ and six as 3+. In the corresponding formalin-hardened materials 3 were classed as 2+ and 10 as 1+.

TABLE 1.—*Distribution of Dopa Reactions in White Patients Whose Skin Was Prepared by Freeze-Drying and by Formalin-Hardening*

Frozen-Dried Skin		Formalin-Treated Skin				
Reaction	No. of Patients	++++	+++	++	+	0
++++	4	0	0	0	3	1
+++	19	0	0	0	11	8
++	11	0	0	0	4	7
+	2	0	0	0	..	2
0	1	0	0	0	..	1
Total	37	0	0	0	18	19

TABLE 2.—*Distribution of Dopa Reactions in Negro Patients Whose Skin Was Prepared by Freeze-Drying and by Formalin-Hardening*

Frozen-Dried Skin		Formalin-Treated Skin				
Reaction	No. of Patients	++++	+++	++	+	0
++++	7	0	0	3	4	0
+++	6	0	0	0	6	0
++	0	0	0	0	0	0
+	0	0	0	0	0	0
0	0	0	0	0	0	0
Total	13	0	0	3	10	0

COMMENT

The difference in the dependability of the dopa reaction by the two methods is best noted in white patients. Of the 37 specimens studied, 36 (97.3%) gave positive dopa reactions with the method reported here, as against 18 (50%) with the technique generally used. Similar differences were noted in the staining intensity of corresponding tissues prepared by the two methods. (Tables 1 and 2 and Figs. 1 and 2). The use of formalin before incubating the specimen in the substrate is responsible for many negative dopa reactions in skin containing melanin. It probably explains Becker's [9] statement that "The chief reliability of the dopa reaction is in its positivity. When negative, it is impossible to determine whether the reaction is really negative or has simply failed to occur because of some undetermined factor in the technique."

Zimmerman [10] believes that the negative dopa reactions which he found in Negro fetuses under 3 months of age are due to the effect of formalin-hardening on the enzymes. Similarly, one might question some of the other published results of negative dopa reactions in preparations that had been treated with formalin. While this study was limited to 50 patients, the results indicate that if melanin is present in the epidermis, the melanocytes in a high proportion of cases will give a positive dopa reaction, if the tissue has not been treated with formalin and if the preparation is satisfactory for histologic study.

SUMMARY AND CONCLUSIONS

Normal skin from 37 white and 13 Negro surgical patients who were chosen at random was stained by Becker's dopa technique. Adjacent skin in each case was frozen-dried, prepared, sectioned, and stained with dopa by a method described in this report. In the tissues from both white and Negro patients the dopa reaction was more sensitive and more reliably positive with our method than with Becker's technique. Positive reactions were obtained in over 97% of tissues from white patients by this method as against 50% by Becker's technique. The melanocytes stained much more intensely in the preparations from frozen-dried than from the formalin-treated tissues. Even 5% formalin for only a half hour decreased the stainability of melanocytes. The method described permits semiquantitative staining of these cells.

Dr. A. A. Zimmerman, Professor of Anatomy, University of Illinois College of Medicine, gave helpful consultations in the preparation of this study.

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Induced Cancer of the Cervix Uteri in the Mouse

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This investigation was made in an attempt to successfully induce and study experimental cancer of the uterine cervix in the mouse. With use of a technique suggested by Murphy, a continuous application of carcinogen was obtained by means of an impregnated thread suspended in the cervical canal.

MATERIALS AND METHODS

A total of 80 female mice of the C3H strain, obtained from the Jackson Memorial Laboratory, were used. They were approximately 12 weeks of age and weighed an average of 18.9 gm. at the onset of the experiment. The mice were kept in an air-conditioned room under constant temperature and humidity, caged in groups of 20 animals, and given a stock diet with tap water ad libitum.

The experimental group included 65 animals, all of which had a carcinogen-impregnated thread suspended in the uterine cervix. A #12 six-cord cotton thread, washed in changes of water, alcohol, and ether, was first cut into suitable lengths. A knot was then formed in the end of each thread by superimposing three simple overhand knots. The knotted ends of the threads were then impregnated for an average distance of 3 mm. with 20-methylcholanthrene and beeswax in a ratio of 1:3, the average content of carcinogen being 1.15 mg. as determined by weight.

The mice were anesthetized by intraperitoneal injection of 2% pentobarbital sodium, and, with use of a clean technique, the carcinogen-impregnated thread was inserted into the cervical os from below by means of a straight blunt-tipped needle so that the knot was positioned in the region of the uterine cervix. The needle was carried upward through one uterine horn and outward through the anterior abdominal wall, using care to avoid perforating the intestine. The thread was then anchored in the abdominal wall by means of a knot which was later buried in the subcutaneous tissues.

The vaginal secretion was examined at frequent intervals in all mice in order to gain some knowledge of the cellular changes occurring in the uterine cervix. Specimens were collected by means of a capillary pipette. A small amount of isotonic saline solution was introduced into the vagina and aspirated through the pipette. The material was expressed onto the surface of a glass slide and distributed as evenly as possible with the pipette. The preparations were fixed in 10% neutral formalin solution and stained with a polychrome technique so that the exfoliated cells might be examined.

After first acquiring knowledge of the cellular changes normally observed throughout the estrus cycle in the mouse, consideration was given to the morphology of the altered cells exfoliated in the presence of induced cancer. A study was made of the cellular preparations in 50 mice. Animals of the present series are included in this group, as well as others from more recent studies. With use of a systematic meander screening pattern designed to cover the entire slide, the characteristics of altered cells were recorded at regular intervals in an attempt to provide a random sampling. The features of 50 altered cells were recorded for each of the 50 specimens.

RESULTS

Of the 15 mice in the control group, 2 died within seven weeks after the operative procedure. At autopsy, in one of these animals there was an acute peritonitis and pneumonitis. The 13 surviving animals were killed at 16 weeks. None showed gross or microscopic evidence of cancer.

There were 65 mice in the experimental group, of which 8 died within seven weeks after the insertion of the carcinogen-

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From the Department of Pathology of the Western Reserve University School of Medicine and the University Hospitals of Cleveland.

impregnated thread. All eight animals had acute peritonitis at autopsy. The 57 surviving animals were either killed when a tumor mass was apparent prior to the termination of the experiment or were arbitrarily killed at 16 weeks.

A total of 11 mice in the experimental group did not have histopathological evidence of invasive cancer at autopsy. Of these, two were killed in the 15th week because of tumor masses which proved to be abscesses on histological examination. The remaining nine mice killed at the termination of the experiment did not have invasive cancer on microscopic examination.

Out of 65 mice in the experimental group, 46 had malignant neoplasms involving the uterine cervix. Prior to the 14th week, there were seven mice killed because of tumor masses which proved to be cancers on gross and microscopic study. As early as seven weeks, two mice had developed cancers. In the 14th and 15th weeks there were 9 and 16 animals, respectively, which were killed because of masses which were ultimately proved to be cancer. In addition, 14 mice killed at the termination of the experiment had developed squamous-cell carcinoma (Fig. 1).

The induced cancers had an average maximum dimension of 1.8 cm., the largest measuring 4.2 cm. in greatest dimension. There was involvement of the uterine cervix in all 46 mice with induced cancer. Extension was noted to the body of the uterus in 43 mice, to parametrial sites in 43 mice, and to the peritoneum in 10 mice. There was involvement of the upper vagina in 30 animals and invasion of the rectum in only 2 mice. An anterior extension of the process was seen in 16 mice. In these the neoplasm extended to involve the skin of the anterior abdominal wall in the site of the buried thread.

The urinary bladder was directly invaded by cancer in only one animal. Dilatation of the urinary bladder resulted from compression of the bladder or urethra in seven mice. In addition, there was notable compression of one or both ureters in 5 animals, and a total of 15 showed some degree of hydronephrosis or hydroureter. The latter animals were included in the 25 mice showing histopathological evidence of pyelonephritis. There was a wide variation in the severity of the inflammatory change occurring in the kidneys and ureters.

Cancer was detected within endothelial-lined spaces in nine mice, while only six showed definite evidence of metastases. The para-aortic lymph nodes were involved in five animals, the cancer being confined to the peripheral sinusoids without complete replacement. The adrenal gland and lung were each involved by metastatic cancer in one animal.

Fig. 1.—Uterus of a mouse with induced cancer. There is involvement of the corpus, cervix, and upper vagina. × 2.

Associated changes of note included dilatation of the uterine cavity and pyometra found in 23 and 10 mice, respectively. This was in part attributed to the obstruction of the cervical canal either by the knot or the tumor mass. An organizing acute peritonitis was present in 18 animals, of which 2 had hepatic abscesses.

On histopathological examination all neoplasms were characterized by changes which warranted their being interpreted as squamous-cell cancers. Fundamentally, the neoplasms were either exophytic in type or were characterized predominantly by invasive growth. The latter group included cancers with broad sheets of cells that encroached upon the surrounding tissues predominantly by compression as well as other cancers with smaller cell cords exhibiting a more marked infiltration of the stroma.

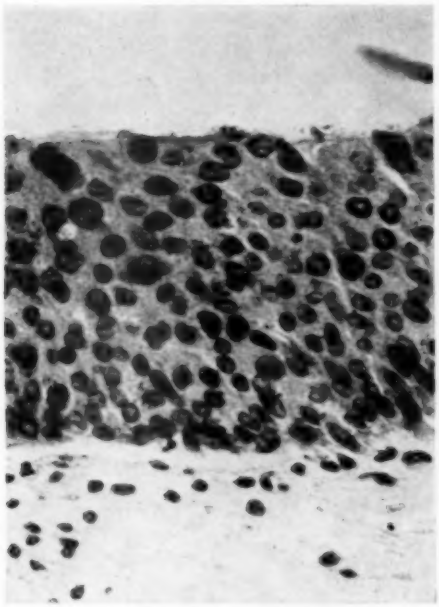

Fig. 2.—Alteration in the cervical mucosa of the mouse which is similar to the precancerous change observed in the human; × 340.

There was considerable variation in the degree of cellular differentiation both in different areas in the same tumor and in comparing one neoplasm with another. Examples of the histopathological changes are shown in Figures 3 and 4. Keratinization was a prominent feature in many cancers but was inconspicuous in others. Similarly some cancers showed a marked anaplasia, while others were made up of relatively uniform and mature cells. One notable variant was characterized by invading masses of cells in which there was a central cavity

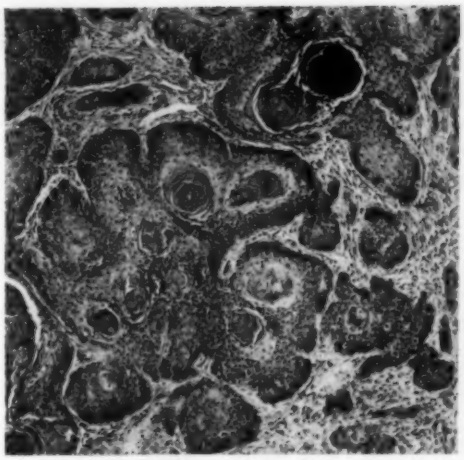

Fig. 3.—Induced cancer of the uterine cervix of the mouse with prominent keratinization; reduced about ⅕ from mag. × 102.

lined by columnar epithelium which was infiltrated by neutrophiles and showed degenerative changes. The columnar epithelium was similar to that normally observed covering the stratified squamous epithelium in the mouse cervix during proestrus and presumably was carried into the stroma by the invading tumor cords.

In addition to the 46 mice showing outspoken cancer, there were 6 animals killed in the 16th week of the experiment that

Fig. 4.—Induced cancer of the uterine cervix of the mouse in which there was extension to the urinary bladder and ureters; reduced about ⅙ from mag. × 120.

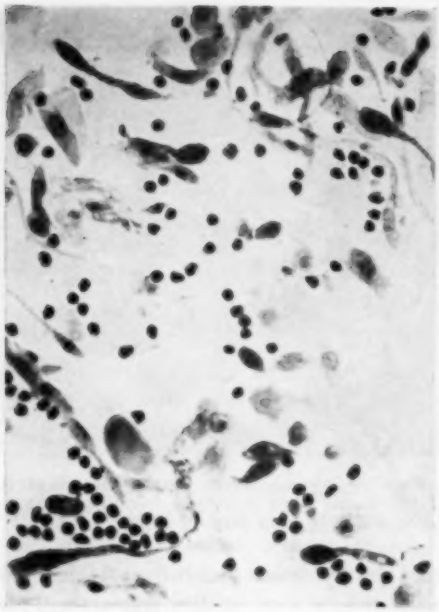

Fig. 5.—Cellular forms observed in the vaginal secretion of a mouse with induced cancer. There is a predominance of elongate and so-called "tadpole" forms. × 134.

Fig. 6.—So-called "tadpole" form observed in the presence of induced cancer in the mouse. Note the delicate cytoplasmic fibril which is in part responsible for the unusual configuration. × 800.

showed significant changes confined alone to the surface epithelium. The histopathological changes were similar to those encountered in surface epithelium adjacent to frank cancer in other mice. The lesions were similar in many respects to the precancerous changes in the human cervix.

There were characteristic cellular changes in the aspirated vaginal fluid from mice with induced cancer. These were readily distinguished from those noted in studying the estrus cycle in the normal mouse and from

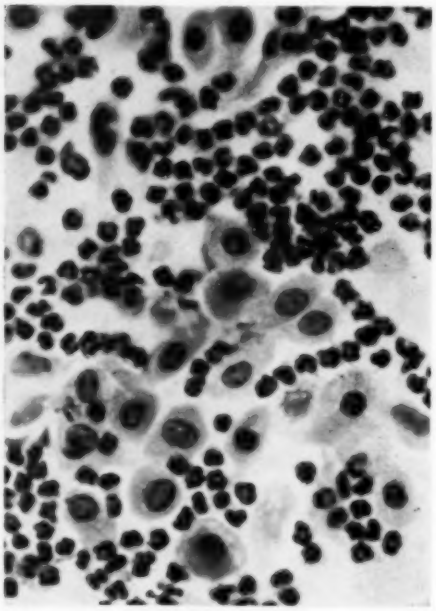

Fig. 7.—Prominent nuclear enlargement, hyperchromatism, and altered chromatin patterns in cells derived from induced cancer of the mouse cervix; × 480.

the cellular changes observed in the control animals.

A varied configuration was observed in the 2500 cells interpreted as being abnormal. Of these, 28.30% were oval, 26.40% were irregular in configuration, 17.36% represented so-called "tadpole" forms, 14.86% were elongate, 8.72% were polyhedral, and 4.36% were more or less round (Figs. 5, 6, and 7).

An acidophilic cytoplasmic staining reaction was noted in 42.72% of the cells, a

basophilic reaction in 57.00%, and an indeterminate staining reaction in 0.28%. Cytoplasmic vacuolation was prominent, being noted in 34.32% of those studied.

The nuclear mass was relatively large in many of the cells recognized as altered elements. The nucleus was oval in 75.00% of the cells, elongate in 14.20%, irregular in 8.32%, and round in only 2.48%. A prominent nuclear hyperchromatism characterized many of the cells. This was associated with a basically coarsely granular nuclear pattern in 96.52% of the cells, while in 3.48% the nuclear chromatin pattern was basically finely granular. There were distinct nucleoli in 10.36% of the cells examined, while chromocenters were more numerous. Multinucleation characterized 7.68% of the cells examined.

Of the 2500 cells studied, 89.24% were isolated in the cellular preparations, while 10.76% of the abnormal cells encountered in the screening patterns were arranged in aggregates.

COMMENT

This study demonstrates that cancer can be readily produced in the mouse cervix by means of a carcinogen-impregnated thread suspended in the cervical canal. Out of 57 mice in the experimental group surviving the operative procedure by more than 7 weeks, 46 developed invasive cancer during the 16-week period of the study.

The latent period was relatively short in using this method for inducing cancer of the uterine cervix, and the number of "takes" in the surviving animals relatively high. Since in some instances the threads could not be identified in the animals killed at 16 weeks without demonstrable cancer, there is reason to believe that the number of "takes" might possibly be increased provided the impregnated threads had remained in position.

Comparable results were reported by Murphy. With the thread technique, Murphy noted 27 cancers in 35 strain-A mice at an average latent period of four and one-half months, while in painting experiments he obtained 13 squamous-cell cancers of the cervix and vagina out of 28 mice at an average latent period of nine and one-half months.

The cancers observed in the present study were similar to those encountered in the human cervix, not only in their histological appearance but also in their mode of extension. There was involvement of the fornices and vaginal wall, parametria, and uterine body, as well as the urinary tract and rectum, and para-aortic lymph nodes. The procedure employed to induce the cancers was in part responsible for the type of extension seen in some animals.

In addition to the 46 mice with outspoken cancer, there were 6 animals killed in the 16th week of the study with significant alterations confined alone to the surface epithelium. In these the mucosal changes were similar in many respects to those regarded as precancerous in the human. The component cells were immature with relatively large and hyperchromatic nuclei. There were alterations in nuclear chromatin, multinucleation, and scattered mitoses. Isolated cell keratinization was observed in some lesions, together with an alteration in the polarity of the component cells. A lesion interpreted as a precancerous change is shown in Figure 2. The number of these lesions was too small to permit any conclusions as to their significance. Comparable changes were not observed in the control animals.

Studies of the cells exfoliated in the presence of experimentally induced cancer are of considerable interest. There are several basic differences between the malignant tumor cells and those normally encountered in the estrus cycle of the mouse. While the normal cells are predominantly isodiametric, pleomorphism is more commonly observed in the cells derived from induced cancer in the uterine cervix of the mouse. Similarly, in many of the malignant tumor cells there was definite nuclear enlargement and outspoken hyperchromatism. These changes were commonly associated with distinctive changes in the nuclear chromatin, i.e., a

more coarsely granular nuclear pattern in contrast to the basically fine granularity of the (normal) interphasic nucleus. Other significant changes in nuclear structure were also observed, although they were inconstant in the cases examined.

The described alterations in the component cells of the vaginal fluid are significantly different from those in the normal. Of the 46 mice with induced cancer in this series, all had cellular changes which were considered to be consistent with those described. Similar changes were not seen in the control animals of this or subsequent series, and in only two animals of the present series were there cellular changes of the type described without proved invasive cancer. Both animals had palpable masses which on the basis of step sections were believed to be of inflammatory origin. On the basis of subsequent studies serial sections must be employed to exclude the presence of invasive cancer in these animals. Thus, in the presence of the described cellular changes in exfoliated cells, there is a high probability that cancer exists in the uterine cervix and, on the other hand, in the absence of these changes it is unlikely that cancer is present.

This work will serve as the basis for further studies on experimental carcinogenesis in the uterine cervix of the mouse.

SUMMARY

Out of 57 C3H mice surviving more than seven weeks, there were 46 in which cancer of the uterine cervix was induced by means of carcinogen-impregnated thread suspended in the cervical canal. In addition, six mice had changes which were similar to those considered to be precancerous in the human.

The cellular elements of the vaginal fluid were studied in order to learn the morphology of the altered cells derived from the induced cancers.

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Periductal Lymphoid Infiltrations in Mammary Tissue

MAURICE M. BLACK, M.D.
and
FRANCIS D. SPEER, M.D., New York

In the course of a study of the stromal changes in breast carcinoma, it was noted that normal-appearing parenchymal ducts were frequently surrounded by lymphocytic aggregates. These findings were of interest in view of our previous observations concerning the prognostic significance of lymphoid infiltrates in breast and gastric carcinoma. It therefore seemed pertinent to determine the frequency and possible significance of the periductal lymphoid infiltrates in control and cancerous breasts. To this end we have studied the cellular reactions in the periductal stroma of mammary tissues removed from patients with fibrocystic disease and in the non-neoplastic portions of breasts with carcinoma. The data indicate that periductular lymphoid infiltrations are commonly observed in the cancerous breasts but are infrequent in cancer-free breasts. Furthermore, such cellular infiltrates in cancerous breasts are distinct from and not necessarily accompanied by lymphocytic infiltrations within the invading tumor.

MATERIALS AND METHODS

Studies were made of the breast tissues from 129 cases of breast carcinoma and 118 cases of fibrocystic disease of the breast. It should be mentioned that the fibrocystic group included structural variations from essentially normal mammary parenchyma to extensive adenosis cystic changes and duct ectasia. For this investigation we employed the slides from the surgical pathology files of the Flower and Fifth Avenue Hospitals, as they were routinely prepared and stained with hematoxylin and eosin. No attempt at selection of cases was made, and no special stains were employed.

The slides from the tumor cases were examined for evidence of lymphoid infiltrations (*a*) within the substance of the invasive tumor, (*b*) in areas where the tumor cells, while recognizable as such, were still in situ, and (*c*) in the regions of normal-appearing parenchymal structures peripheral to the tumor. The control breast sections were also carefully searched for evidence of cellular infiltrates. In almost all cases a minimum of three sections of breast tissue were available for study.

The character and components of the cellular reactions were noted and the intensity graded from 0 to 4+. A 4+ reaction was indicative of a dense focalization of cellular infiltrate intimately associated with the structure under observation. It is not implied, however, that such reactions were found throughout the sections. It should be noted that the lymphoid infiltrations under discussion are distinct from chronic inflammatory infiltrations and are not associated with stromal scarring or vascular proliferation.

RESULTS

Control Series.—In the Table we have indicated the incidence of the varying degrees of periductular lymphoid infiltration observed in the sections from the control breasts. It will be noted that marked (≧3+) lymphoid infiltrates were infrequently observed. This low incidence was

Per Cent Incidence of Varying Degrees of Periductal Lymphocytic Infiltration in Breast Parenchyma from Control and Cancer Cases

Group	No. Cases	0	1	2	3	4
Control.....	118	80%	6%	3%	1%	1%
Cancer.....	129	47%	11%	24%	11%	7%

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Department of Pathology, New York Medical College, Flower and Fifth Avenue Hospitals.

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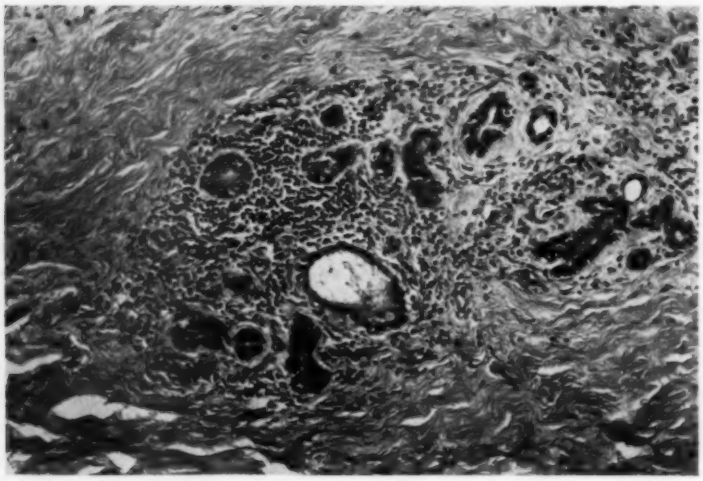


Fig. 1.—Lymphoid infiltrate around mammary ducts in a case with minimal cystic disease; reduced about ⅓ from mag. × 100.

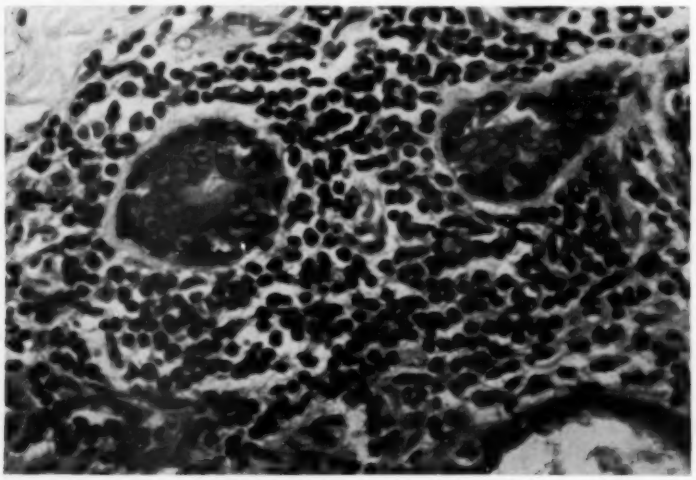


Fig. 2.—Higher magnification of Fig. 1. Note the nuclear and nucleolar prominence in the ducts surrounded by the lymphoid infiltrate. Reduced about ⅓ from mag. × 440.

all the more striking when one considers that the majority of the cases showed florid adenosis, microcystic and macrocystic changes, areas of stromal scarring, and chronic inflammatory changes coincident to duct ectasia. It would, therefore, appear that the periductular lymphoid accumulations are different from the chronic inflammatory stromal changes which are commonly associated with duct ectasis.[3] It should also be mentioned that the epithelial cells of the ducts associated with the lymphocytic reactions tended to show nuclear prominence. In Figures 1 and 2 we have depicted a focal area of periductular lymphoid infiltrate observed in a breast biopsy from a case of fibrocystic disease. It will be noted that the infiltrate in this instance is concentrated around a duct showing distinct cytological atypia in the form of anisonucleosis, high nucleocytoplasmic ratios, and very prominent nucleoli.

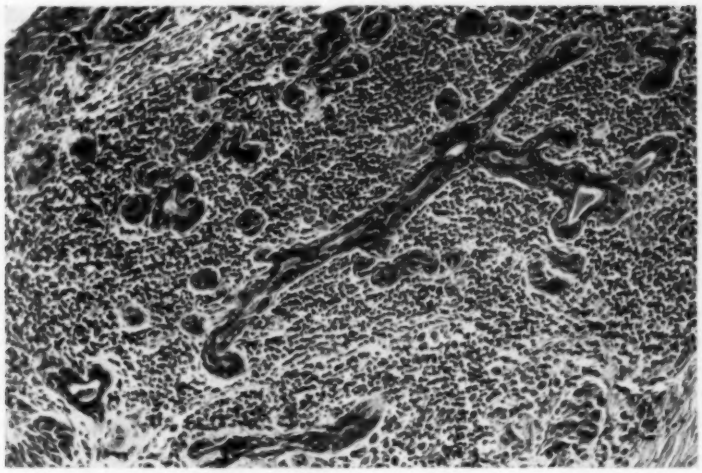


Fig. 3.—Periductular lymphoid infiltrate in a control area of a cancerous breast. Note the foci of invading cancer cells at the edges. The cancer tissue per se was devoid of any stromal reaction. Reduced about ⅓ from mag. × 100.

Fig. 4.—Area of intraductal papillary carcinoma with periductal lymphoid infiltrate. Widely invasive carcinoma without any lymphoid infiltrate was found in other sections. Reduced about ⅓ from mag. × 100.

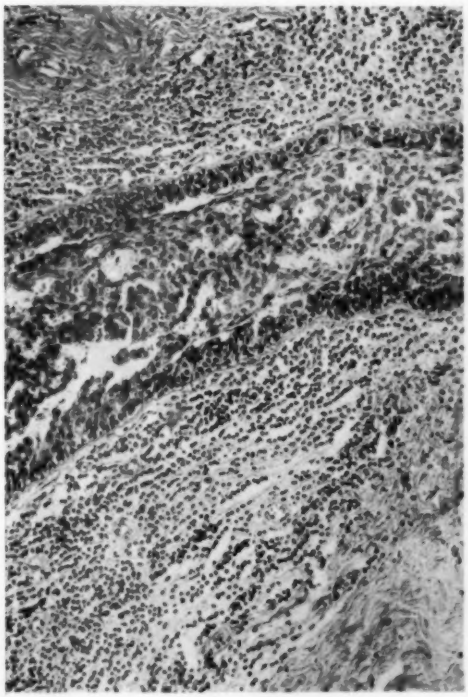
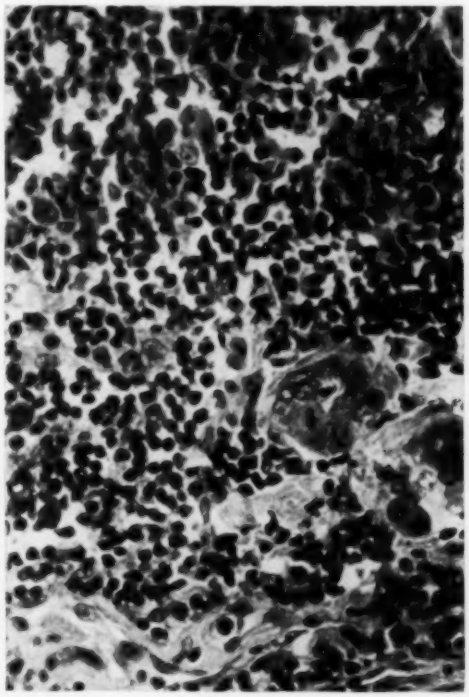


Fig. 5.—In situ malignant changes in ducts of breast showing widely invasive carcinoma in adjacent areas. Despite the marked lymphoid infiltrate in this area the tumor proper was devoid of such reactions. Reduced about ⅓ from mag. × 440.



Breast Cancer Series.—The incidence of the various degrees of periductular lymphoid infiltrate found in the noncancerous areas in this series is indicated in the Table. In contrast to the control series, marked lymphoid infiltrations ($\geq 3+$) were found in 18%, and moderately intense reactions ($2+$) were observed in an additional 24% of the cases (Fig. 3).

It should be emphasized, however, that such reactions were found in the parenchymal areas in close proximity to the carcinomas, whereas the distant parenchymal tissues were rarely involved with such changes.

Lymphoid infiltrations were also noted around ducts in which the epithelium showed clear-cut evidence of malignant changes, i.e., in situ areas of carcinomas (Figs. 4 and 5). In such cases lymphoid infiltrates were also found around benign-appearing duct structures.

A statistical comparison was made of the incidence of the various degrees of periductal infiltrate in the two series, utilizing the χ^2 technique. Utilizing four degrees of freedom, it was found that the probability that the observed differences were the result of chance variation was less than 1 in 100.

Despite the relative frequency (42%) of peripheral lymphoid infiltrations, such infiltrations within the cancer tissues were uncommon in this series ($<5\%$). Thus the peripheral lymphoid infiltrations are not necessarily or even commonly associated with infiltrations within the invading tumor. However, the converse is not true. Of 13 cases having moderate or marked lymphocytic infiltrations within the primary tumor, peripheral infiltrates $\geq 2+$ were found in 11 cases (85%). This incidence is significantly higher than that found in the control series or in the unselected cancer series ($P<0.01$).

COMMENT

The ability to invade and proliferate in heterotopic sites is one of the most constant and characteristic features of malignant neoplasia. Green has suggested that this property might be connected with an antigenic change in the tumor cell proteins, so that "tumor recognition" was lost.[4] He proposed that the critical essence of malignant transformations was this change in antigenicity. If this is so, then the possibility exists that the antigenic transformation as it occurs in the preinvasive stage would create a cell antigenically foreign to its site of origin. It might then be expected that some type of tissue reactions would be found in relation to areas of such in situ antigenic changes. In this connection it is pertinent to note that lymphoid reactions are found in the area of rejected tumor implants.[5] Furthermore, heterologous transplantation is made possible by the use of powerful lymphocytolytic agents.[6]

In the present study the pertinent findings included the following: (*a*) Periductular lymphoid aggregations were found in about 40% of the cancer cases but were rare in the control series; (*b*) lymphoid aggregates occurred around ducts whose epithelium appeared normal, hyperplastic, or clearly neoplastic; (*c*) periductal lymphoid aggregates were far more frequent than lymphoid infiltrations within the tumor proper; (*d*) in those cases with lymphocytic infiltrations within the tumor, periductal lymphoid aggregates were frequently observed (85%).

It should also be mentioned that we have observed lymphocytic reactions in the region of origin of diverse carcinomas and melanomas in numerous sites. The details of these observations will be reported separately. However, it is pertinent to note that Couperus and Rucker found lymphocytic reactions at the site of origin of more than 90% of a large series of melanomas.[7]

It would, therefore, appear that epithelial structures undergoing malignant changes go through biochemical (antigenic) changes which precede the microscopic structural alterations characteristically associated with malignant transformation. The existence of this prestructural change is seemingly associated with focalized lymphoid infiltrations. However, one must also consider that in only a minority of cancer cases did the

lymphoid infiltrations accompany the invading cancer cells. The lack of such infiltrations within the tumor proper is conceivably the result of (*a*) complete loss of identity proteins of the cancer cell, so that heterotopic localization incites no reaction, and/or (*b*) diminution or loss of host reactivity to antigenic stimulation. The latter possibility is supported by reports of anergic states in cancer patients.*

In essence, then, the results of the present study are consistent with the view that the initial changes in carcinogenesis include alterations of the antigenic character of the cells so as to favor heterotopic survival. Such alterations appear to be opposed by biological reactions associated with lymphoid infiltrations. Where the antigenic transformation is complete, the tumor cells invade without evidence of local tissue opposition of the type visualized by lymphoid infiltrates.

The observations that some invasive carcinomas are associated with lymphoid infiltrations suggest that complete heterotopic compatibility is not always achieved. In this regard, it is of great significance that such cases are characterized by superior survivals.†

While the above hypothesis may not be the only possible explanation of the observed data, it does appear to be internally consistent and would seem to be of value as a basis for further experimental study of the complex biological phenomena involved in the origin and behavior of malignant neoplasia.

* References 8 and 9.

† References 1, 2, 10, and 11.

SUMMARY

A study was made of the cellular reactions in the periductular stroma of mammary tissues from patients with fibrocystic disease and in the non-neoplastic portions of breasts with carcinoma. It was observed that (*a*) periductular lymphoid aggregations were found in about 40% of the cancer cases but were rare in the control series; (*b*) lymphoid aggregates occurred around ducts whose epithelium appeared normal, hyperplastic, or clearly neoplastic; (*c*) periductal lymphoid aggregates were far more frequent than lymphoid infiltrations within the tumor proper; (*d*) in those cases with lymphocytic infiltrations within the tumor, periductal lymphoid aggregates were frequently observed (85%).

These findings were discussed in terms of antigenic changes in cancer tissues.

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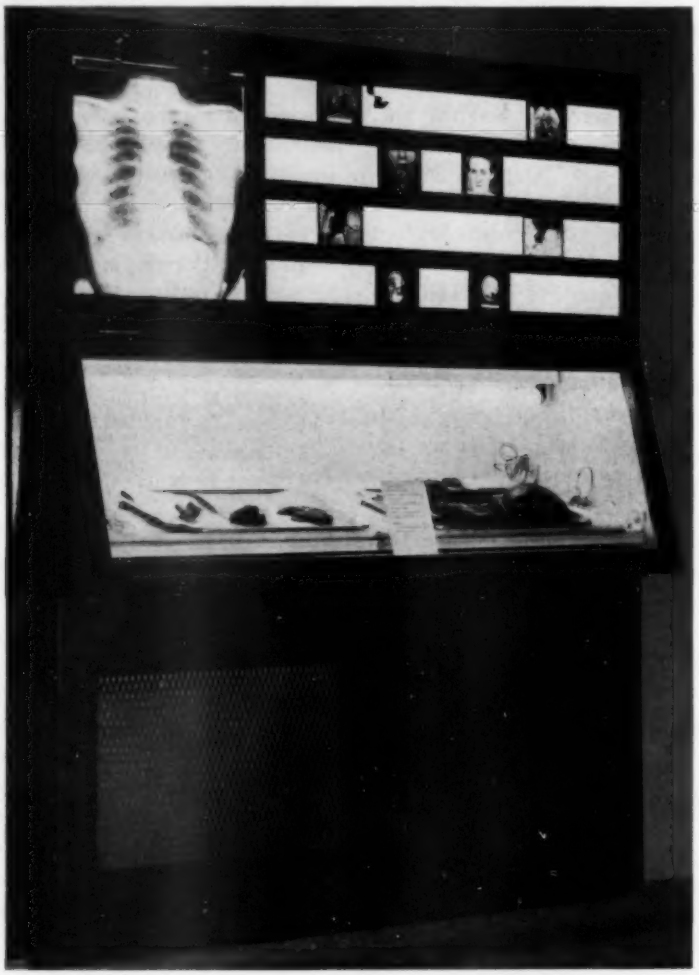
Laboratory Methods and Technical Notes

A REFRIGERATED DISPLAY CABINET

SAMUEL HANSON, M.D., Edmonton, Alta., Canada

A specially constructed cabinet for the display of fresh gross specimens from the operating rooms and the necropsy room has been in use at our hospital for about a year (Figure).



This cabinet is built into the wall of the laboratory corridor where the corridor adjoins the entrance into the operating room suite. In this area there is practically no patient traffic. Members of the medical staff on their way to the operating rooms or to the doctors' sitting room pass by the cabinet.

Fresh material is placed on display as it becomes available. In our 400-bed hospital something new is available at least every other day. A short history and descriptive note is fastened to the front glass panel over each specimen. The fresh specimen area is refrigerated by a Dole Freezing Plate maintained at a constant temperature of 30 F by a sealed compression unit. This unit is located beneath the refrigerated display case. A standard

Submitted for publication June 28, 1955.

Director of Laboratories, Edmonton General Hospital.

temperature control with a low-temperature expansion valve is used.

Above the refrigerated area we have incorporated a standard roentgen-ray view box where pertinent radiographs are placed on display. We find these especially valuable when displaying gastrointestinal and pulmonary material from cases which have been previously investigated by roentgen ray. Adjacent to the roentgen-ray view box is a lantern-slide display area where are placed photomicrographs of material taken from the gross specimens displayed below or photographs of any interesting material one may wish to display.

We have found that the material so displayed is of keen interest not only to medical staff members but to graduate and undergraduate technologists and nurses. It allows us to make better use of specimens for teaching at all levels. It has several advantages over permanently mounted glass-jar or watch-glass specimens, and it is superior to color photographs alone. Its constantly changing display maintains a high level of interest.

Fresh gross material keeps without deterioration for about a week. We have found it advisable to cover the specimens with moist paper towels overnight.

News and Comment

PERSONAL

Retirement of Dr. Esmond R. Long.—Dr. Esmond R. Long retired, on July 1, as Director of the Henry Phipps Institute for the Study, Prevention, and Treatment of Tuberculosis of the University of Pennsylvania, and as Director of Medical Research for the National Tuberculosis Association. Dr. and Mrs. Long plan to make their home in Pedlar Mills, Va., where he will continue to devote his time to research and writing.

Appointment of Col. Dwight M. Kuhns.—Col. Dwight M. Kuhns (MC), U. S. A., has been appointed Army Deputy Director of the Armed Forces Institute of Pathology. Since January, 1953, Col. Kuhns has been Chief, Pathology and Allied Sciences Division, Office of the Surgeon General, Department of the Army.

Cancer Society Award to Dr. Fred W. Stewart.—Dr. Fred W. Stewart, Chief of the Department of Pathology at the Memorial Center for Cancer and Allied Diseases, New York, has received the Bronze Medal of the American Cancer Society for 1954, for his contributions to the control of cancer.

ANNOUNCEMENTS

Annual Meeting of American Public Health Association.—The 83d Annual Meeting of the American Public Health Association will be held in the Municipal Auditorium at Kansas City, Mo., Nov. 14-18. Papers of interest in the field of cancer research will be presented by E. V. Cowdry, Harold L. Stewart, Francisco Duran-Reynals, R. Lee Clark, Jr., Raymond F. Kaiser, and others.

Latin-American Congress of Pathology.—The First Latin-American Congress of Pathology will be held in México, D. F., from Dec. 11 to 17. Further information may be obtained by writing to Dr. Ruy Perez-Tamayo, Laboratorio de Anatomia Pathologica, Instituto N. de Cardiologia, Avenida Cuauhtemoc 300, México 7, D. F.

Books

Pathogenesis of Poliomyelitis. By Harold K. Farber, M.D. Price, $5.00. Pp. 176, with 22 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1955.

This monograph treats the various aspects of the topic under consideration at about the appropriate length for the interested observer of the progress of experimental work and recent speculations in this field. As is the custom in such a monograph, the author has tended to emphasize his own conclusions regarding such controversial points as the pathogenetic importance of viremia, route of invasion of the central nervous system, and the pathogenesis of symptomatology. It is clear that many of the conclusions stated are not acceptable to other workers in the field, and one would feel somewhat more comfortable if some of the points summated in the "Recapitulation" were more tentatively advanced.

The discussion and illustration of infection of peripheral ganglia is especially well done. It would have been useful, perhaps, if illustrations and more detailed discussion of the central nervous system pathology of poliomyelitis were included.

The author has a straightforward and lucid style and the book is well printed on glossy paper.

Christopher's Minor Surgery. By Alton Ochsner, M.D., F.A.C.S., and Michael E. DeBakey, M.D., F.A.C.S. Price, $9.00. Pp. 547, with 251 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1955.

The Seventh Edition of "Christopher's Minor Surgery" is an entirely new book both in authorship and in organization. It represents an excellent summary of considered, established opinions concerning diagnosis and treatment of surgical conditions which do not require hospitalization. Alton Ochsner and Michael E. DeBakey, the editors, have enlisted the cooperation of nineteen of their associates, so that each subject is presented by a specialist in the field. Each chapter, written by one man, is a well-organized, critical, lucid presentation of his experience and concludes with a few well-chosen references to significant recent research and pertinent reviews in the field. There is sufficient detail to make the book of practical value for the neophyte and sufficient wisdom to render it of interest to the experienced surgeon. Recognition of surgical disease and office diagnostic procedures such as proctoscopy receive healthy emphasis. The chapter arrangement is according to systems. There is an excellent chapter on "The Surgical Resident," by Frederick F. Boyce. The printing and illustration are excellent. This volume is reasonably priced and will be of outstanding value to all students and practicing doctors, regardless of specialty.

Shearer's Manual of Human Dissection. Edited by Charles E. Tobin, Ph.D. Price, $6.00. Pp. 287, with 79 illustrations. McGraw-Hill Book Company, Inc., 330 W. 42d St., New York 36, 1955.

This book is the third edition in eighteen years of a manual for dissection in gross human anatomy. The directions provided serve well the purpose of the book as set forth by Dr. Shearer in the preface to the first edition: "The aim of the present work is to point out to the inexperienced dissector what structures he can reasonably be expected to see in the time at his disposal, and to give directions, with as little excess verbiage as possible, for the procedures which should be followed in the demonstration of these structures."

This third edition of this manual has been revised by Dr. Charles E. Tobin. The revisions include different and more effective dissection procedures for the head, neck, and perineum and the inclusion of certain structures which had been omitted hitherto.

As in the second edition, the important structures are set forth in boldface type, and the printing is quite clear and easy to read, an important consideration for dissecting room use. There are 79 illustrations, and they are generally of good quality, with few exceptions, the exceptions being certain figures which are either too small to be readily referred to or are representations of rather uncomplicated regions which present no difficulty for the average student. Since the concurrent use of a standard text and/or atlas is necessary, further presentation of such relatively simple areas is not essential. On the whole, however, the illustrations are good and add definitely to the value of the book as a practical dissection manual.

The arrangement of the regional dissections is such that this manual may be used effectively over a rather wide range of sequential approaches as the individual instructor may desire.

This volume is a well-organized, versatile, and clearly presented manual of dissection which will materially aid both instructor and student in the most effective use of the limited time now generally available for gross anatomy. Instructors and students alike should welcome the appearance of the third edition of this popular manual.

Animal Pathology. By Russell A. Runnells. Price, $8.50. Pp. 718. The Iowa State College Press, Press Building, Ames, Iowa, 1954.

This book is designed as a pathology text for veterinary students. The section on general pathology is based on the conventional concepts of human pathology but is oriented to animal disease. The chapters on systemic pathology cover briefly many diseases of several species with emphasis on animals of commercial importance (horse, cow, swine) and with a fair amount of material on the dog. The book is soundly written and is a valuable source of information for medical investigators who are interested in comparative pathology or who are using any of these species as laboratory animals.

Pathology of the Dog and Cat: The Genitourinary System, with Clinical Considerations. By Frank Bloom. Price, $12. Pp. 463, with 312 illustrations. American Veterinary Publications, Inc., Box 872, Evanston, Ill., 1954.

This detailed monograph on the genitourinary diseases of the dog and cat is based on study of a large amount of biopsy, surgical, and autopsy material obtained by the author in his veterinary practice. In addition to providing a reference work for veterinarians, the author has attempted (with considerable success) to produce a treatise that will be useful for workers in medical research. The section on kidney disease is excellent. The material on several tumors is of great interest. The gross and microscopic photographs are uniformly of good quality. The author states that many of the lesions dealt with have not previously been described but often does not indicate in the text which ones these are. In certain situations this information would be helpful.

Tumors of the Soft Tissues. By Arthur Purdy Stout, M.D. Price, $2.00. Pp. 138, with 78 illustrations and 6 color plates. Armed Forces Institute of Pathology, Washington 25, D. C., 1953.

This fascicle continues the same format and same high standards of reproduction that have characterized previous sections of the "Atlas of Tumor Pathology." No finer authority could have contributed this fascicle, for certainly the work of Arthur Purdy Stout has made him outstanding in this field. In his presentation Dr. Stout makes full use of many of his earlier works. This monograph considers first the benign soft tissue tumors as fibromatoses, myxomatoses, xanthomatoses, lipomatoses, and the various angiomatoses. Muscle tumors and tumors often associated with bone are discussed. In turn, the malignant counterparts of these tumors are described. Also, brief mention is made of malignant lymphomas and tumors of the reticuloendothelial system in order to give proper emphasis to some of these common malignant lesions. An excellent bibliography completes the fascicle.

The Harvey Lectures, Series 49 (1953-1954). Delivered under the auspices of the Harvey Society of New York. Price, $7.50. Pp. 244. Academic Press, Inc., 125 E. 23d St., New York 10, 1955.

This book includes the Harvey Lectures delivered under the auspices of the Harvey Society of New York for 1953-1954. The lecturers in this period were Claude Fromageot, Hans H. Weber, David Nachmansohn, Paul Klemperer, Rollin D. Hotchkiss, Britton Chance, Albert L. Lehninger, Linus Pauling, and James D. Hardy. The subjects included "The Metabolism of Sulfur and Its Relations to General Metabolism," "Adenosine Triphosphate and Motility of Living Systems," "Metabolism and Function of the Nerve Cell," "The Significance of the Intermediate Substances of the Connective Tissue in Human Disease," "The Genetic Chemistry of the Pneumococcal Transformations," "Enzymes in Action in Living Cells: The Steady State of Reduced Pyridine Nucleotides," "Oxidative Phosphorylation," "Abnormality of Hemoglobin Molecules in Hereditary Hemolytic Anemias," and "Control of Heat Loss and Heat

Production in Physiologic Temperature Regulation." It would obviously be impossible to review these lectures in detail. The readers of the A. M. A. ARCHIVES OF PATHOLOGY, however, will be particularly interested in the excellent discussion by Paul Klemperer on "The Significance of the Intermediate Substances of the Connective Tissue in Human Disease."

Progress in Allergy, IV. Edited by Paul Kallós. Price, $20.00. Pp. 520, with 149 figures and 63 tables. Little, Brown & Company, 34 Beacon St., Boston 6, 1955.

This volume, of over five hundred pages, contains discussions as follows:

Histology of Allergic and Related Lesions, by M. G. Bohrod

Group-Sensitization to Compounds of Quinone Structure and Its Biochemical Basis: Role of These Substances in Cancer, by R. L. Mayer

Delayed Hypersensitivities, by S. Raffel

The Biosynthesis of Adrenal Steroids, by Pincus

Functional Interrelationships Between the Anterior Pituitary and Adrenal Cortex in Intermediary Metabolism, by F. L. Engel

The Mechanisms of Action of Adrenocortical Hormones in Allergy, by T. F. Dougherty

New Contributions to Experimental Asthma, by I. Noelpp-Eschenhagen and B. Noelpp

Respiratory Allergy to Fungus Spores, by K. Maunsell

The articles by Pincus, Engel, and Dougherty, in particular, are noteworthy in that they attempt to bring into focus the relationship of hormonal factors in the field of allergy. The review by Engel is a masterly one, and it alone is documented with 325 references. As Engel says, "The presentation of an exhaustive review on the anterior pituitary and adrenal cortex and intermediary metabolism in a publication devoted to allergy is a reflection of the coming age of the science of endocrinology." Similarly, in the article by Dougherty, which is documented with 195 references, the many confusions and controversial aspects with respect to the immunological significance of the adrenocortical hormones are considered. The introductory chapter by Kallós carries with it 129 references. These points are emphasized because the articles have obviously been prepared with thoroughness. This volume should be of interest to all who are concerned with the problems of immunity and allergy.

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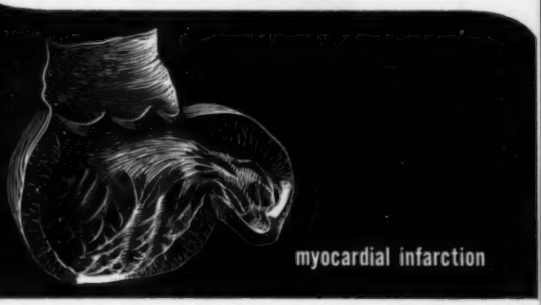
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